Molecular and Genomic Analysis of Clinical Multidrug-Resistant Coagulase-Negative Staphylococci from the uMgungundlovu District in the KwaZulu-Natal Province, South Africa.

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JONATHAN ASANTE

BPharm (KNUST, GHANA), MPharm (UKZN, SA)

Molecular and Genomic Analysis of Clinical Multidrug-Resistant Coagulase-Negative Staphylococci from the uMgungundlovu District in the KwaZulu-Natal Province, South Africa.

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JONATHAN ASANTE

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This is a thesis in which the chapters are written as a set of discrete research manuscripts, published or intended for submission to peer-reviewed journals, with a general introduction and final summary.

This is to certify that the content of this thesis is the original research work of Mr. Jonathan Asante, carried out under our supervision at the Antimicrobial Research Unit (ARU), Discipline of Pharmaceutical Sciences, School of Health Sciences, Westville Campus, University of KwaZulu-Natal (UKZN), Durban, South Africa.

Superviso[redacted]

Signed: -[redacted]---- Name: Professor Sabiha. Y. Essack Date: 14-04-2021

Co-Supervisor:

Signed: --[redacted]--- Name: Dr. Daniel G. Amoako Date: 12-04-2021

Supervisor: [redacted]

Signed: ---- Name: Dr. Akebe L.K. Abia Date: *12-04-2021*

DECLARATION

I, **Jonathan Asante**, declare that

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DEDICATION

This work is dedicated to my family and all loved ones whose support has brought me this far.

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3. **Jonathan Asante,** Bakoena A. Hetsa**,** Daniel G. Amoako, Akebe L.K. Abia, Linda A. Bester, and Sabiha Y. Essack. Genomic analysis of multidrug-resistant *Staphylococcus epidermidis* isolates from clinical sources in the KwaZulu-Natal Province, South Africa (Submitted to **Frontiers Microbiology** [Manuscript number: 656306]).

LIST OF PEER-REVIEWED PUBLICATIONS RELATED TO THIS THESIS

1. Adzitey F, **Asante J**, Kumalo HM, Khan RB, Somboro AM, Amoako DG. Genomic Investigation into the Virulome, Pathogenicity, Stress Response Factors, Clonal Lineages, and Phylogenetic Relationship of *Escherichia coli* Strains Isolated from Meat Sources in Ghana. Genes 2020;11(12):1504 (published)
2. **Asante J**, Osei Sekyere J. Understanding antimicrobial discovery and resistance from a metagenomic and meta-transcriptomic perspective: advances and applications. Environmental Microbiology Reports 2019;11(2):62-86. doi:10.1111/1758-2229.12735 (published)
3. **Asante J**, Noreddin A, Zowalaty MEE. Systematic Review of Important Bacterial Zoonoses in Africa in the Last Decade in Light of the 'One Health' Concept. Pathogens 2019;8(2):50 doi:10.3390/pathogens8020050 (published)
4. Osei Sekyere J, **Asante J**. Emerging mechanisms of antimicrobial resistance in bacteria and fungi: advances in the era of genomics. Future Microbiology 2018;13(2):241-62. Doi: 10.2217/fmb-2017-0172 (published).

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LIST OF ABBREVIATIONS AND ACRONYMS

ACME	Arginine Catabolic Mobile Element
AMR	Antimicrobial Resistance
ARG	Antibiotic Resistance Gene
ATCC	American Type Culture Collection
CARD	Comprehensive Antibiotic Resistance Database
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-Negative Staphylococci
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeat
DNA	Deoxyribonucleic Acid
ICU	Intensive Care Unit
IS	Insertion Sequence
KZN	KwaZulu-Natal
MARI	Multiple Antibiotic Resistance Index
MDR	Multidrug Resistance
MGE	Mobile Genetic Element
MIC	Minimum Inhibitory Concentration
MLS_B	Macrolide-Lincosamide-Streptogramin B
MLST	Multilocus Sequence Typing
MRCoNS	Methicillin-Resistant Coagulase-Negative Staphylococci
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MRSE	Methicillin-Resistant *Staphylococcus epidermidis*
MSCRAMM	Microbial Surface Components Recognising Adhesive Matrix Molecule
NICU	Neonatal Intensive Care Unit
PATRIC	Pathosystems Resource Integration Center
PBP2a	Penicillin-Binding Protein 2a
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
RAST	Rapid Annotation using Subsystem Technology
R-M System	Restriction Modification System
RNA	Ribonucleic Acid

SCC	Staphylococcal Cassette Chromosome
SNP	Single Nucleotide Polymorphism
WGS	Whole-Genome Sequencing
WHO	World Health Organization

ABSTRACT

Coagulase-negative staphylococci (CoNS) are among the most commonly recovered bacteria in clinical specimens. They are usually colonisers (commensals) of the skin and nasal passages and considered contaminants of microbial cultures. However, they have been recognised as emerging pathogens, frequently causing opportunistic infections. The frequent use of indwelling medical devices and long-term hospitalisation present an increased risk of exposure to CoNS, resulting in infections usually caused by multidrug-resistant pathogens.

Few studies focus on CoNS, including characterisation of their mechanisms of resistance, virulence, and persistence. Therefore, this study describes the molecular and genomic profiles of clinical CoNS from public sector hospitals in the uMgungundlovu District in KwaZulu-Natal, South Africa.

Eighty-nine clinical CoNS isolates collected from three hospitals within the uMgungundlovu District between October 2019 and February 2020, constituted the sample. Isolates were speciated using the Vitek 2 system. Antibiotic susceptibility testing was done against a panel of 20 antibiotics according to Clinical and Laboratory Standards Institute (CLSI) guidelines using the Kirby-Bauer disk-diffusion method and minimum inhibitory concentration (MIC) was determined using the broth microdilution method for penicillin G, cefoxitin, ceftaroline, ciprofloxacin, moxifloxacin, azithromycin, erythromycin, gentamicin, amikacin, chloramphenicol, tetracycline, doxycycline, teicoplanin, tigecycline, linezolid, clindamycin, rifampicin, sulphamethoxazole/trimethoprim, nitrofurantoin and vancomycin. PCR was used to detect the presence of the *mecA* gene to confirm phenotypic methicillin resistance.

Based on their resistance profiles, a sub-sample of isolates were subjected to whole-genome sequencing (Illumina MiSeq) to ascertain the resistome, virulome, mobilome,

clonality and phylogenomic relationships using bioinformatic tools. The SPAdes software was used for the assembly of the raw reads. ResFinder 4.1 and CARD were used to identify antibiotic resistance genes in the isolates, while the virulence factor database (VFDB), Center for Genomic Epidemiology‘s MLST 2.0 server and MobileElementFinder v1.0.3 were used to identify virulence genes, sequence types and mobile genetic elements, respectively. Mutations in fluoroquinolone and rifampicin resistance genes were identified by manual curation using BLASTn alignment which was also used to determine the genetic environment of the resistance genes.

S. epidermidis was the most abundant CoNS species isolated. Phenotypic methicillin-resistance was detected in 76.4% (n=68) of isolates, 92.6% (n=63) of which were genotypically confirmed by PCR. Multidrug resistance (MDR) was observed in 76.4% (n=68) of isolates, with 51 antibiograms observed. The resistance genes *mecA*, *blaZ, erm(A), erm(B), erm(C), msr(A), aac(6')-aph(2'')* and *fosB,* among others, were detected and corroborated the observed phenotypes. Molecular mechanisms of resistance to tigecycline, teicoplanin, linezolid and nitrofurantoin were not detected even though some isolates were resistant to them. There was no association between ARG type and hospital/department. The *ica* operon known to facilitate biofilm formation was detected in 7/16 isolates sequenced. Known and putatively novel mutations in the *gyrA*, *parC, parE* and *rpoB* genes were also detected for fluoroquinolone- and rifampicin-resistant isolates. Prediction of isolates' pathogenicity towards human hosts yielded a high average probability score (Pscore ≈ 0.936), which, together with the several virulence genes detected (including *atl, ebh, clfA, ebp, icaA, icaB,icaC*), support their pathogenic potential to humans.

Seven MLST types were found, while the community-acquired SCC*mec* type IV was the most common SCC*mec* type detected. Mobile genetic elements (MGEs) haboured by isolates included plasmid replicon Rep10 and insertion sequence IS256. Defense systems such as arginine catabolic mobile element (type I and III), CRISPR system (16), and the restriction-modification system (type II) were detected. Genetic analysis showed that resistance genes were frequently bracketed by MGEs such as transposons (such as Tn554) and insertion sequences (such as IS257 and IS1182) that facilitated their mobility. Phylogenetic studies showed that the distribution of genes did not coincide with the phylogenetic clades. Despite the relatedness of isolates (clades A and B), there is still considerable variation within individual strains that can facilitate adaptation to local environments. The isolates exhibited several permutations and combinations of ARGs, virulence genes and MGEs, pointing to a complex milieu of mobilized antibiotic resistance and pathogenic characteristics in clonal and multiclonal strains. The study necessitates surveillance of CoNS as emerging pathogens.

CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Antibiotic resistance has become a subject of global interest, especially as the use of antibiotics continues to rise in both clinical and veterinary practice. Bacteria adapt to the effects of antibiotics in several ways to enable them to survive in therapeutic concentrations of antibiotics (Osei Sekyere and Asante, 2018). Thus, infections caused by pathogenic bacteria have become increasingly difficult to treat due to the various antibiotic resistance mechanisms deployed by bacteria to evade the effects of antibiotics (Holmes et al., 2015, Osei Sekyere and Asante, 2018).

Also implicated in antibiotic resistance are the so-called persister cells, which can survive under low metabolic states in the presence of antibiotics. Persister cells are so named because they can persist under the stress of antibiotics due to their metabolic inactivity (Lee et al., 2016, Osei Sekyere and Asante, 2018). The nondividing nature of persister cells is central to their ability to withstand the lethal effects of antibiotics because binding to drug target sites will not be able to alter cells' activities. Persister cells are thought to be able to transfer resistance traits to non-persisters, rendering them resistant (Lewis, 2010, Wood et al., 2013).

Coagulase-negative staphylococci (CoNS), major components of the normal flora of the skin and mucous membranes, are amongst the most frequently recovered bacterial species in microbiology laboratories, where they are mostly regarded as contaminants in microbiological cultures (Asante et al., 2020). The pathogenic potential of CoNS has been underestimated for a long period. However, they have been recognised as opportunistic pathogens, often causing multidrug-resistant infections (Asante et al., 2020, Perez et al., 2020). Notably, their acquisition of methicillin resistance, mediated by *mecA* gene (encoding alternative form of penicillin-binding protein, PBP2a), is a cause of concern due to limited treatment options for such strains, and that comes at increased cost in lower-middle-income countries (Asante et al., 2020, Asante et al., 2019).

CoNS (including *Staphylococcus epidermidis*, *S. haemolyticus* and *S. saprophyticus*) are increasingly implicated in significant infections, including urinary tract infections, endocarditis, surgical site infections, and prosthetic joint infections (Becker et al., 2014, Rogers et al., 2009). They are frequently associated with bacteremia in patients habouring central and peripheral venous catheters, orthopaedic prostheses, pacemakers and infections involving biofilm formation on inserted biomaterials (Asante et al., 2020, Becker et al., 2014, García et al., 2004). Biofilm formation, which facilitates the adherence of CoNS to surfaces of biomaterials and host tissues, is one of the main virulence mechanisms deployed by CoNS to establish infection. Biofilms play the function of providing a protective barrier against antibiotics and to evade the immune response (Goetz et al., 2017).

Studies characterising CoNS, including their virulence and resistance mechanisms in South Africa, are relatively few, especially in clinical settings with scanty descriptions of their molecular epidemiology. Even fewer are the number of studies that have conducted whole-genome sequencing (WGS) studies into clinical CoNS isolates. This study thus describes the genomic profile of CoNS species obtained from hospitals within the KwaZulu-Natal Province in South Africa, using WGS and bioinformatics analysis to help fill the information gap.

2.0 Literature review

This section provides a brief overview of the literature on antibiotic resistance, the epidemiology of resistance genes, the molecular mechanisms of resistance in Gram-positive bacteria, CoNS and the role of WGS and bioinformatics analysis in antimicrobial resistance (AMR) research. The literature review is further elaborated in Chapter Two in the form of a paper entitled “Review of Clinically and Epidemiologically Relevant Coagulase-Negative

Staphylococci in Africa," published in Microbial Drug Resistance. This review article discusses studies from Africa where CoNS species were suspected as the cause of various infections, focusing on identification/laboratory detection, their clinical relevance, transmission, antibiotic susceptibility, typing, and treatment.

2.1 The threat of antibiotic resistance

The discovery and subsequent improvements of antibiotics in the 20th century gave a significant boost to the fight against infectious diseases, revolutionizing the therapy of infectious diseases and improving health and quality of life through the prevention and treatment of infectious diseases (Asante and Osei Sekyere, 2019). Indeed, so bright were the prospects that it led to the thinking that premature death due to infectious agents would be confined to the past. However, the introduction of antibiotics into clinical practice has been associated with the selection of antibiotic-resistant strains, thus rendering the drugs progressively ineffective in treating several infections (Brown and Wright, 2016, Osei Sekyere and Asante, 2018).

The ability of bacteria to resist the effects of antibiotics that they were previously susceptible to, is called antibiotic resistance (Davies and Davies, 2010). The ability of resistant bacteria to share resistance genes with susceptible bacteria, thereby making them also resistant, contributes to the spread of antibiotic resistance in a given niche. It is estimated that mortality due to AMR will reach 10 million by the year 2050 if measures are not put in place to stem the tide (O'Neill, 2014).

Microorganisms can evolve through epigenetic mechanisms and genetic variations in response to selection pressure. Antimicrobial resistance (AMR) continues to compromise the effective prevention and treatment of several infections caused by bacteria, fungi, viruses, and parasites, posing challenges to treating infectious diseases, making treatment costly or even impossible

(Brown and Wright, 2016, Osei Sekyere and Asante, 2018). A surge in the global morbidity and mortality as a result of infectious diseases has occurred partly due to antimicrobial resistance (Brown and Wright, 2016).

There are documented reports of resistance to all known antibiotics currently in use, including reserve antibiotics such as tigecycline, vancomycin, carbapenems, ceftaroline and colistin (Osei Sekyere, 2016, World Health Organization, 2017). This leaves clinicians with severely reduced options in treating infectious diseases, especially those caused by multidrug-resistant pathogens. It is thus imperative to safeguard the use of antibiotics to preserve the already limited options available as the development of new antibiotics has stalled over the past 30 years (Asante and Osei Sekyere, 2019, Brown and Wright, 2016).

2.2 Known mechanisms of antibiotic resistance

Antibiotic resistance in bacteria can be intrinsically expressed or may be acquired. Soil bacteria are known to produce antibiotics to ward off natural competition from neighbouring bacterial species (Pehrsson et al., 2013). These bacteria usually develop resistance to protect them against the antibiotics produced by themselves, as do the neighbouring bacteria to escape the effects of the naturally produced antibiotics (Asante and Osei Sekyere, 2019). The resistance mechanisms developed, as a result, can be transferred to other non-resistant bacteria through mobile genetic elements (MGEs) such as plasmids, transposons, integrons, integrative and conjugative elements, insertion sequence common regions and gene cassettes. This process, referred to as horizontal gene transfer (HGT), helps to facilitate the spread of antibiotic resistance genes in bacteria (Stokes and Gillings, 2011).

Intrinsic resistance refers to the innate ability of bacteria to resist the effects of an antimicrobial agent through its innate functional or structural characteristics, without the need for mutation or gain of extra genes (Davies and Davies, 2010). During antibiotic therapy, susceptible microbes are inhibited/killed, leaving the resistant ones to proliferate. This allows for the predominance of resistant bacteria within a given niche. Selection pressure is exerted by any condition that allows bacteria with intrinsic or acquired resistance to survive and multiply (Holmes et al., 2016). Generally, bacteria mediate intrinsic resistance by two major mechanisms, i.e., by membrane impermeability and inaccessibility, and extrusion of antibiotics through chromosomally-encoded efflux pumps. For example, due to their thin peptidoglycan layer and extra lipopolysaccharide-containing outer membrane, Gram-negative bacteria are resistant to the glycopeptide vancomycin, which is too large a molecule to enter the cell. As well, in some intrinsically resistant bacteria, the porin size and chemical properties restrict the entry of certain antibiotics (Cag et al., 2016).

Bacteria have well-differentiated mechanisms by which they evade and develop resistance to antibiotics. Generally, bacteria elude the effects of antibiotics by three primary mechanisms, which usually function synchronously with one another (Cag et al., 2016). These mechanisms include inactivation of the drug by enzymes such as β-lactamases and aminoglycoside/fluoroquinolone acetyltransferases; target modification as is the case with DNA gyrase and topoisomerase for fluoroquinolone resistance and decreased uptake of the drug through efflux upregulation and porin downregulation (Cag et al., 2016).

Enzymatic inactivation of drugs is observed in β-lactams, where β-lactamases can bind to and inactivate them. When β-lactams are hydrolysed, they form open rings that are ineffective in binding to their target, penicillin-binding proteins (PBPs) (Cag et al., 2016). Other enzymes, in contrast to β-lactamases, alter antibiotics by adding chemical groups that decrease antibiotics' affinity to their target molecules. For example, the antibiotic chloramphenicol may be altered by the enzyme chloramphenicol acetyltransferase (CAT), which changes chloramphenicol to an inactive mono- or diacetate form (Zienkiewicz et al., 2017). Resistance to aminoglycosides has developed in different bacteria species through the production of aminoglycoside-modifying enzymes (AMEs), which can acetylate, phosphorylate, or adenylylate the drug. AMEs inactivate aminoglycosides by mimicking their RNA targets and replacing them (Chee-Sanford et al., 2009, Davies and Davies, 2010).

Bacteria can replace or modify molecules targeted by antibiotics, making the drug molecules unable to bind to their intended target, thus evading the biocidal/biostatic effects of antibiotics (Cag et al., 2016). Such mechanisms are observed in quinolones, macrolides, polymyxins and β-lactams. Notably, bacteria develop resistance to β-lactams by altering their primary targets, PBPs (Asante et al., 2019). Quinolone resistance may occur as a result of modifications to DNA gyrase (Gyr A) (Liu et al., 2012), while the activity of macrolides may be stifled by the methylation of its target site viz., the 23s rRNA (Asante et al., 2019). Resistance to vancomycin, which for a long time was considered the agent "of last resort" for MRSA infections, may occur through modifications in the drug target D-Ala–D-Ala terminus of peptidoglycan, allowing for bacterial cell wall synthesis even in the presence of vancomycin (Cui et al., 2003, Cui et al., 2006).

Bacteria may prevent the buildup of antibiotics on their target molecules by reducing drug absorption into cells, increasing drug expulsion from the cell, or by utilizing both mechanisms concurrently (Delcour, 2009). Efflux mechanisms actively pump out antibiotics from within the cell to prevent their accumulation or interaction with their target-site molecule(s) (Osei Sekyere et al., 2016). Efflux molecules may work in concert with porin modifications to intensify the expulsion of antibiotics from within the bacterial cell. Porins are bacterial cell membrane protein channels (Poole, 2005).

2.3 Epidemiology of resistance genes and molecular mechanisms of resistance in Gram-positive bacteria

The first report of resistance in penicillin to a penicillin-inactivating enzyme, even before it became available clinically (Abraham and Chain, 1940), signaled a severe setback in antimicrobial chemotherapy. Resistance to all classes of antibiotics has been observed in clinically relevant bacteria, including resistance to aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin), β-lactams (penicillins, cephalosporins, carbapenems, monobactams, cephems), quinolones (nalidixic acid and later generations of fluoroquinolones), glycopeptides (vancomycin, teicoplanin), sulphonamides, trimethoprim, rifampicin, tetracyclines (tetracycline, doxycycline, minocycline, oxytetracycline) polymyxins (colistin, polymyxin B), phenicols (chloramphenicol, thiamphenicol, florfenicol), glycylcyclines (tigecycline), and macrolides (azithromycin, erythromycin). This observed resistance has resulted from the use of these antibiotics in the clinical, veterinary and agricultural settings (Hancock, 2005, Laxminarayan et al., 2013).

Resistance to β-lactams among Gram-positive bacteria arises through mutations in PBPs and other non-PBP related genes. These kinds of mutations and changes in PBPs decrease the affinity of PBPs for β-lactam antibiotics, including the anti-Gram-positive cephalosporins (Asante et al., 2019, Odonkor et al., 2012). For instance, when methicillin-susceptible *Staphylococcus aureus* strains acquire the *mecA* gene, which encodes penicillin-binding protein 2a (PBP 2a), they become methicillin resistant. PBP 2a is different from the usual penicillin-binding proteins and does not bind to methicillin or other β-lactam antibiotics at its active site (Amoako et al., 2019b, Asante et al., 2019). The *mecA* gene is borne on the mobile element referred to as the staphylococcal cassette chromosome SCC*mec* in staphylococci. Thus, resistance to β-lactams may be mediated by both chromosomal mutations in PBPs and/or

MGEs such as SCC*mec* in staphylococci and plasmid-borne PBP in enterococci, pneumococci and streptococci (Amoako et al., 2019a, Asante et al., 2019).

In both Gram-positive and Gram-negative bacteria, resistance to tetracyclines, fluoroquinolones and aminoglycosides are transferred by plasmids bearing integron- and transposon-mobilised resistance genes (Cag et al., 2016). In Gram-positive bacteria, however, resistance to fluoroquinolones is mainly due to chromosomal mutations in topoisomerase genes (*grlA* and *grlB*) and gyrase genes (*gyrA* and *gyrB*) and overexpression of efflux pumps, as seen in NorA efflux pump and its related counterpart, pmrA, which mediate resistance to fluoroquinolones in *S. aureus* and *S. pneumonia* respectively (Berger-Bächi, 2002).

Generally, resistance to newer agents such as glycylcyclines (tigecycline), oxazolidinones (linezolid), lipopeptides (daptomycin), ramoplanin, ketolides (telithromycin), streptogramins (quinupristin/dalfopristin), ceftaroline, telavancin, diphenyl glycopeptides (oritavancin and dalbavancin) and the older cationic antimicrobial peptides (polymyxins) is minimal and limited to isolated cases in parts of the globe (Hancock, 2005, Osei Sekyere, 2016). Indeed, most of these newer agents are very effective against resistant/difficult-to-treat Gram-positive related infections such as MRSA and vancomycin-resistant *Enterococci* (VREs). Thus, there are more reserve treatment options for recalcitrant Gram-positive related infections than Gram-negative ones (Hancock, 2005).

2.4 *Staphylococcus* species and CoNS

The term staphylococcus was first described in 1883 by Ogston, for the micrococci group causing pus formation and inflammation (Sugawara and Nikaido, 2014). *Staphylococcus* species are spherical bacteria that can occur singly, in pairs, tetrads, or more commonly in grapelike clusters. They are Gram-positive cocci ranging in diameter from 0.5-1µm. Staphylococci are widespread in nature and are aerobic, non-spore-forming, nonmotile, facultative anaerobic bacteria, able to survive under diverse conditions on environmental surfaces. They are regular inhabitants of the skin, skin glands and mucous membranes, especially the nasal cavities. Moreover, they can be found symbiotically in the mouth, intestines, upper respiratory and genitourinary tracts. However, they can assume pathogenic roles when they gain access to other sites within the body through broken skin and mucosal membranes (Fontana and Favaro, 2018).

Staphylococci (especially *S. aureus*) may cause mild local infections of the skin such as impetigo, furunculosis and cellulitis. If left untreated, these infections can proceed to life-threatening infections such as osteomyelitis, bacteremia, meningitis, scalded skin syndrome, pneumonia, and encephalitis by spreading to various organs through blood vessels and neighbouring tissues (Fontana and Favaro, 2018). Owing to its ability to produce many virulence and pathogenic determinants, *S. aureus* is the most pathogenic among the staphylococci species (Bien et al., 2011). Staphylococci possess a vast arsenal of virulence genes, which may be species- or strain-specific, facilitating adherence to surfaces, persistence, colonisation, invasion, toxicity, and evasion of the intrinsic and adaptive immune response (Becker et al., 2014).

Generally, compared with *S. aureus,* the virulence of CoNS is under-researched, save for biofilm formation by *S. epidermidis* (Becker et al., 2014). Biofilm-associated infections, including those caused by CoNS, are highly resistant to antibiotics due to slime production, with biofilm-producing strains about 1000-fold more resistant than their planktonic counterparts (Fontana and Favaro, 2018).

CoNS such as *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus* are of concern as opportunistic pathogens in human and animal hosts. Of the CoNS, *S. lugdunensis* is noted for its ability to cause purulent infections (Fontana and Favaro, 2018). The epidemiology and clinical relevance of CoNS have been discussed extensively in chapter 2 of this thesis.

In summary, staphylococci are cause for concern both as community and nosocomial pathogens, with an emerging role in human and animal diseases, requiring the attention of microbiologists and clinicians. There is a paucity of information on the molecular epidemiology of drug-resistant CoNS in South Africa and Africa. Furthermore, studies involving the use of WGS on CoNS isolates are rare in Africa, thus creating an information gap on the molecular mechanisms of resistance and virulence, their mobilisation through MGEs as well as strain types and phylogenies. This study provides a perspective from Northdale, Edendale and Grey's hospitals in the uMgungundlovu District in the KwaZulu-Natal Province in South Africa.

3.0 Aim

To describe the molecular epidemiology, phenotypes and genotypes of coagulase-negative staphylococci clinical isolates from uMgungundlovu District, KwaZulu-Natal (KZN), South Africa.

3.1 Specific objectives

1. To ascertain the incidence of CoNS from hospitals in the uMgungudlovu District of the KwaZulu-Natal Province from blood cultures routinely processed by the central microbiology laboratory using culture and biochemical techniques.
2. To speciate the CoNS using the automated VITEK 2 system.
3. To determine the susceptibility profile of CoNS isolates against a CLSI-recommended antibiotic panel using the Kirby-Bauer disk-diffusion and MIC (broth microdilution) methods as appropriate for the antibiotics: penicillin G, cefoxitin, ceftaroline, ciprofloxacin, moxifloxacin, azithromycin, erythromycin, gentamicin, amikacin,

chloramphenicol, tetracycline, doxycycline, teicoplanin, tigecycline, linezolid, clindamycin, rifampicin, sulphamethoxazole/trimethoprim, nitrofurantoin and vancomycin.

4. To determine the ability of CoNS isolates to form biofilm using the quantitative (tissue culture plate) method.
5. To describe the clinical and epidemiological relevance of CoNS by way of a literature review.
6. To identify and characterize antibiotic-resistance and virulence genes in CoNS, their associated MGEs and their genetic support/environment using whole genome sequencing and bioinformatics tools such as ResFinder, Comprehensive Antibiotic Resistance Database (CARD), VirulenceFinder, virulence factor database (VFDB), BacWGSTdb, Rapid Annotation using Subsystem Technology (RAST), SCCmecFinder, PlasmidFinder and MobileElementFinder.
7. To determine the clonal relatedness and phylogeny of isolates using whole-genome sequencing and bioinformatics tools such as CSIPhylogeny, Figtree and Phandango to compare and contrast study strains with others.

4.0 Synopsis of methodology

4.1 Ethical considerations

The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference: BREC/00001302/2020) as a sub-study of the overarching research programme on Antibiotic Resistance and One Health (Reference: BCA444/16). The study was originally envisaged as a comparison between pig and clinical isolates as part of the overarching research project stated above. Pig samples were obtained and processed as part of the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) project and the National Health Laboratory Service (NHLS) was asked to collect and store CoNS isolates while an application to expand the bacterial species investigated in the overarching research programme from *S. aureus* only to *Staphylococcus spp*. was made to BREC. Very few CoNS samples were recovered from the study on pigs, precluding comparison. The study thus took a purely clinical focus investigating the molecular epidemiology of CoNS from hospitals in the uMgungundlovu district.

4.2 General methodology

The study describes the molecular epidemiology, phenotypic and genotypic characteristics of CoNS isolated from hospitals in the uMgungundlovu district of the KwaZulu-Natal Province, South Africa. Presumptive CoNS isolates (from blood cultures) were obtained from routine blood cultures processed by central microbiology laboratory that receives samples from hospitals in the district. Isolates were collected over five months from October 2019 to February 2020 by the central laboratory and stored in the Antimicrobial Research Unit at the University of KwaZulu-Natal. Isolates were initially characterised and identified by culture and biochemical tests and speciated using the automated VITEK 2 system (BioMérieux, Marcy-L'Etoile, France). Coagulase activity or its absence was determined using the Staphaurex™ Latex Agglutination Test and polymerase chain reaction (PCR) determination of *mecA* gene was used to confirm methicillin resistance. The antibiotic susceptibility profiles of isolates were determined using the Kirby-Bauer disk-diffusion method against a panel of 20 antibiotics. The biofilm-forming ability of CoNS isolates was quantitatively determined using the tissue culture plate assay method.

The resistome, virulome, mobilome, clonality and phylogeny of circulating CoNS clones were ascertained using WGS and bioinformatic tools. Comparative genomics of CoNS was done to assess dominant endemic clones spreading in the hospital setting.

5.0 Outline of the thesis

This study is presented in the form of journal articles and manuscripts and comprises the following five chapters:

- **Chapter 2**. Manuscript 1: Review of Clinically and Epidemiologically Relevant Coagulase-Negative Staphylococci in Africa. Microbial Drug Resistance, February 2019; https://www.liebertpub.com/doi/10.1089/mdr.2019.0381. This review discusses studies from Africa where CoNS species were suspected as the cause of various infections, focusing on identification/laboratory detection, their clinical relevance, transmission, antibiotic susceptibility, typing, and treatment.
- **Chapter 3**. Manuscript 2: Multidrug-resistant coagulase-negative staphylococci from the uMgungundlovu District of KwaZulu-Natal Province in South Africa: Emerging Pathogens. This manuscript, submitted to the Journal Antibiotics, describes the types and susceptibility profiles of clinical CoNS isolates in the KZN Province in South Africa.
- **Chapter 4**. Manuscript 3: Genomic analysis of multidrug-resistant *Staphylococcus epidermidis* isolates from clinical sources in the KwaZulu-Natal Province, South Africa. This manuscript submitted to Frontiers Microbiology, describes the antibiotic resistome, mobilome, virulome and phylogenic analysis of clinical methicillin-resistant *Staphylococcus epidermidis* in the KwaZulu-Natal Province, South Africa, using WGS.

- **Chapter** 5. Conclusion. This chapter provides a summary of the work and the significance thereof and includes limitations of the study and recommendations for future research.

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CHAPTER 2 – MANUSCRIPT 1

REVIEW OF CLINICALLY AND EPIDEMIOLOGICALLY RELEVANT COAGULASE-NEGATIVE STAPHYLOCOCCI IN AFRICA.

Author contributions

- **Jonathan Asante**, as the principal investigator, co-conceptualized the study, undertook the literature search, screened the abstracts and full texts, collated all references and drafted the manuscript.
- Daniel G. Amoako, as the co-supervisor, co-conceptualized the study, undertook screening of texts and critical revision of the manuscript.
- Akebe, L. K. Abia, as the co-supervisor, co-conceptualized the study, undertook screening of texts and critical revision of manuscripts.
- Anou M. Somboro undertook critical revision of the manuscript
- Usha Govinden undertook critical revision of the manuscript.
- Linda A. Bester undertook critical revision of the manuscript.
- Sabiha Y. Essack undertook critical revision of the manuscript.

Objective(s) met: This paper addresses objective **5** (i.e., to describe the clinical and epidemiological relevance of CoNS by way of a literature review within the context of Africa.

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Review of Clinically and Epidemiologically Relevant Coagulase-Negative Staphylococci in Africa

Jonathan Asante,[1,2] Daniel G. Amoako,[1] Akebe L.K. Abia,[1] Anou M. Somboro,[2,3]
Usha Govinden,[1] Linda A. Bester,[3] and Sabiha Y. Essack[1]

Coagulase-negative staphylococci (CoNS) have engendered substantial interest in recent years as pathogenic causes of infections in both human and veterinary medicine, especially in the immunocompromised, critically ill, long-term hospitalized and in those harboring invasive medical devices such as catheters. They have been implicated in infections such as urinary tract infections, bloodstream infections, and invasive device-related infections, and are responsible for substantial economic losses in livestock production. The advancement of diagnostic techniques has increased our understanding of their molecular mechanisms of pathogenicity, even though distinguishing between innocuousness and pathogenicity is still challenging.
The incidence of CoNS varied across the continent in humans and animals (mainly cattle), ranging from 6% to 68% in suspected human infections and from 3% to 61.7% in suspected animal infections, distributed across different geographic locations. Furthermore, there were varying antibiotic resistance patterns observed in CoNS isolates, with high methicillin resistance in some cases, leading to crossresistance against many antibiotics. *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus* were most commonly reported in studies herein reviewed, while the enterotoxin C gene, *atl* E gene, *ica* gene, and hemolysin virulence factors were linked with enhanced pathogenicity. Advancement in identification and typing methods, including whole genome sequencing, virulence screening, and the assessment of the immune status of subjects in studies will help to thoroughly assess the true pathogenic potential of isolated CoNS species in developing countries. Careful antibiotic stewardship guidelines should be followed due to the ability of CoNS to develop multidrug resistance.

Keywords: coagulase-negative staphylococci, Africa, antimicrobial resistance, epidemiology, pathogenicity

Introduction

Coagulase-negative staphylococci (CoNS) are increasingly becoming a global public health threat due to increased antibiotic resistance and invasive surgical procedures, which increase the risk of exposure.[1] Even though CoNS are usually considered innocuous, they are frequently associated with nosocomial infections, leading to greater interest in them as relevant pathogens rather than mere contaminants.[1,2] Bacterial eye infections,[3] prosthetic joint infections,[4] urinary tract infections (UTIs) especially among immunocompromised patients,[5] surgical site infections,[6] infective endocarditis[7] and cattle mastitis[8] are some conditions that have been associated with CoNS.[9]

Because CoNS usually form part of the microbiota of the skin and mucous membranes, proper differentiation between clinically significant and contaminant bacteria is important in bacterial etiology of suspected infections. *Staphylococcus epidermidis* is the most frequently encountered CoNS species in laboratories; notwithstanding other clinically relevant CoNS species, such as *Staphylococcus saprophyticus, Staphylococcus haemolyticus, Staphylococcus schleifieri, Staphylococcus xylosus, Staphylococcus hominis* and *Staphylococcus lugdunensis* that are known to cause infections in humans.[1]

It is known that the formation of biofilm facilitates the pathogenicity of CoNS by enhancing their ability to adhere to surfaces of invasive devices such as pacemakers, catheters, and prosthetic heart valves as well as smooth plastic and tissue surfaces.[10] This mechanism is vital for the persistence of CoNS as biofilm-producing strains are characterized by greater resistance to antibiotics. Indeed, most invasive CoNS strains, which display biofilm formation, also show multidrug resistance, with >80% of them being methicillin resistant.[11]

Associated with the pathogenicity of CoNS species is the possession of a host of virulence factors, encoded by specific genes that allow the pathogen to establish infection within the host. The *atl* E gene encoding the vitronectin-binding cell surface protein, which facilitates primary attachment and the

[1]Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu Natal, Durban, South Africa.
[2]School of Laboratory Medicine and Medical Sciences and [3]Biomedical Research Unit, University of KwaZulu Natal, Durban, South Africa.

ica gene, encoding biofilm formation are examples of virulence genes possessed by pathogenic strains of CoNS.[12] *S. haemolyticus*, *S. epidermidis*, and *S. warneri* have been found to produce hemolysins, which lyse susceptible cells to cause leakage of important molecules.[1]

CoNS, like *S. aureus*, have the ability to develop antibiotic resistance, increasing the cost of treatment and contributing to significant morbidity and mortality.[13,14] Resistance to commonly used antibiotic classes such as macrolides, aminoglycosides, and to a last resort, antibiotics, such as the glycopeptides, has increasingly been reported in CoNS species.[15–17] The sustainable effectiveness of glycopeptides against such resistant strains has become topical since the first recorded cases of teicoplanin resistance in methicillin-resistant coagulase-negative staphylococci (MR-CoNS) in the United States and the United Kingdom.[18] Since then, glycopeptide-resistant CoNS in patients on prolonged vancomycin treatment have been variously reported, thus limiting therapeutic options.[19]

CoNS have a particularly high resistance against methicillin (70%–80%) as indicated by various reports in the Canada, United States, and Latin America.[20,21] CoNS possess a wide range of multidrug-resistant determinants mostly on plasmids that mediate resistance to some major antibiotics and can be transferred between different staphylococcal species such as *S. intermedius* and *S. aureus*.[19] For instance, horizontal transmission of the *mecA* gene (which mediates methicillin resistance and is carried by the mobile genetic element *SCC*mec) to other staphylococcal species has been suggested to be a common occurrence.[22]

As at 2018, there were 54 species and 28 subspecies comprising the genus staphylococcus,[23] whereas 41 CoNS species have been documented as of July 2018.[24]

CoNS are not only problematic pathogens in human medicine, but also cause several infections in animals, particularly in livestock production with resultant economic losses. Globally, CoNS are a known leading cause of mastitis, particularly in Europe.[25] Having been considered opportunistic pathogens, which cause mild mastitis that usually remains subclinical, their importance in intramammary infections (IMIs) is increasing as CoNS species have been frequently isolated from most IMIs.[26] Also, no <24 CoNS species have been identified in cases involving bovine mastitis.[27] Genetic typing methods, such as *SCC*mec typing and multilocus sequence typing (MLST), are essential to relate circulating clones/types to pathogenicity.[28]

Concerning CoNS in Africa, there is a dearth of information about their prevalence, epidemiology, role, and antibacterial susceptibility in human and animal (mainly livestock) diseases. Thus, this review seeks to gather information concerning clinically relevant CoNS species in humans and animals in Africa within the last decade. We herein discuss studies from Africa where CoNS species were suspected as the cause of various infections, focusing on identification/laboratory detection, their clinical relevance, transmission, antibiotic susceptibility, typing, and treatment.

Identification/laboratory detection of CoNS

It is important to distinguish between coagulase-positive staphylococci and CoNS as a basic principle, a practice carried out in most laboratories by the slide and tube coagulase tests. Identification down to species level is important particularly for species suspected to cause infections and informs accurate clinical decision making by clinicians. Correct speciation of CoNS is important in detecting exact causes of infection in nosocomial setting in particular, where appropriate, dependable, inexpensive, and fast identification methods are required for effective diagnoses and prompt treatment.[22] This is mostly achieved by biochemical tests, including commercially available kits.[29] Due to the similar biochemical characteristics exhibited by various CoNS species and species heterogeneity, correct identification by biochemical tests is often challenging. Furthermore, biochemical methods are laborious.[26]

Speciation with biochemical methods is gradually being replaced by molecular methods such as polymerase chain reaction (PCR) and spectrometric methods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).[29] Thus, molecular methods which offer a higher level of precision in identification up to species and subspecies level are advocated where appropriate. However, with molecular methods, where sequence data analysis is involved, there may be challenges with sequence data analysis when poor quality sequence data are deposited into databases.[29]

Mostly, species identification is not carried out and they are frequently reported as a general group, thus limiting information available on their epidemiology and downplaying the actual influence of the various, particularly nonabundant species.[30] MALDI-TOF MS as an identification technique also has a useful application in toxin detection and has been shown to exhibit high accuracy and reproducibility when used to identify CoNS.[26] Other strengths of MALDI-TOF MS lie in its quick turnaround times and low operating costs. However, the initial cost of the instrument is expensive and cannot be afforded by many laboratories, especially in low- and middle-income countries. Furthermore, MALDI-TOF has been reported as unable to detect rare species in close to 50% of cases.[31]

In such cases where rare species are suspected, sequencing of phylogenetically important target genes such as the 16S rRNA gene can prove useful.[31] Indeed, amplification and sequencing of target housekeeping genes such as elongation factor Tu (*tuf*) gene, superoxide dismutase (*sodA*), RNA polymerase B (*rpoB*), and 16S rDNA gene have been shown to assist in discriminating between *Staphylococcus* species and subspecies with varying levels of discriminatory power.[32,33] Molecular methods such as PCR affords higher accuracy, typeability, and reproducibility and are therefore recommended in CoNS speciation.[26,34]

More than half of studies considered in this review used basic culture and standard biochemical tests in identification, hence did not have the wherewithal to identify CoNS to the species level. Multiplex PCR, MALDI-TOF MS, and *tuf* gene sequencing were used for the speciation of CoNS by Schmidt *et al.* while investigating distribution and antimicrobial susceptibility profile of *Staphylococcus* species isolated from cattle with IMIs and humans working in close contact with them.[26] The multiple approach to pathogen identification improves the accuracy of identification up to species level.

With the decreasing costs of next-generation sequencing techniques, there could be a shift from phenotypic- to genome-based diagnoses of infections. The shift is a major boost in the search for known and emerging resistance determinants because whole genome sequencing (WGS) provides all the information required to make informed diagnostic decisions, including the species of the offending pathogen, antibiotic resistance genes (ARGs), evolution, and spread of pathogens.[35] Furthermore, WGS furnishes researchers with additional data not currently provided by routine diagnostic methods or even by molecular techniques such as PCR. WGS can be used to predict antibiotic resistance phenotypes by investigating resistance determinants at the genomic level, thus helping clinicians to make rapid clinical decisions to improve patient care.[34,35] Although, molecular methods such as PCR have been used with great success, WGS, by looking at the entire genome offers an improvement in explaining the genetic and epigenetic mechanisms of observed phenotypic resistance. For example, the observation of phenotypic resistance without the detection of classical or known resistance genes by PCR is solved by WGS, by looking at the entire genome, including genetic changes in the genome which may contribute to the observed resistance.[35] Furthermore, the computational sequence querying used by WGS may be more sensitive than primers used in PCR. The challenges, however, lie in the analysis and interpretation of results, the lack of universality of data analysis platforms, and the large computer memory sizes required for the processing and storage of genomic data.[36]

Epidemiology and clinical relevance

The skin as a barrier provides a protective function. It is inhabited by a plethora of varied microorganisms with CoNS being a commensal of the skin and mucosal microbiota of humans and animals.[37] Studies of the skin metagenome have buttressed the long-known assertion that *Staphylococcus* species have a preference for areas of higher humidity such as the plantar foot region, axillae, and umbilicus.[38] The *S. epidermidis* group (consisting of *S. epidermidis* and *S. haemolyticus*) are the most commonly isolated CoNS species in humans, usually found in moist areas of the skin.[37]

A great challenge when dealing with clinical CoNS is to determine their clinical relevance. Clinicians and microbiologists are frequently faced with determining whether retrieved CoNS are contaminants that intrude during sampling or sample processing, are regular skin or mucous membrane commensals, or are clinically relevant.[30] Because many CoNS species form part of the skin and mucous membrane microbiota, the line between innocuousness and pathogenicity may be indistinct and comes down to virulence strategies employed by the various species as well as host defense mechanisms. Having been considered largely nonpathogenic, the pathogenicity of CoNS had been acknowledged by the 1980s, although there were not enough advanced techniques to delineate their molecular mechanisms.[30]

The significance of CoNS in causing infections is highly recognized in certain vulnerable populations such as the immunocompromised, preterm newborns, the elderly, critically ill, and long-term hospitalized patients as well those with invasive devices.[39] A high number of CoNS in samples may be attributed to contamination that occur during sampling, in which case techniques to minimize contamination are required. However, CoNS may be the true cause of infection and need to be assessed. The ability of commensals to cause infections differ.[40]

The difficulty in determining the pathogenicity of most CoNS samples, including clinical samples, is due partly to poorly defined virulence factors in these strains even though the possession of some genetic markers could prove important in distinguishing between potentially virulent and saprophytic/contaminating strains of CoNS.[19] The possession for example of virulence factors, such as Panton–Valentine leukocidin or toxic shock syndrome toxin 1 and enterotoxins could be associated with increased pathogenicity of CoNS.[41] The CoNS do not possess as many virulence factors as *S. aureus*, reflected in the different spectra of diseases they respectively cause.[41] The detection of phenol-soluble modulins in *S. epidermidis* was found to contribute to endemicity, invasiveness, and persistence in hospital environments[42] and CoNS sepsis, especially when methicillin resistant.[43]

In a study investigating factors that contribute to the pathogenicity of CoNS from clinical sources in comparison with community CoNS strains, it was found that 25% of strains produced toxins that induced hemolysis in 50% of human red blood cells within 1 hour, with some 3% showing lytic activity on human polymorphonuclear cells after 30 minutes.[44] The staphylococcal enterotoxin C gene was detected in 9% of *S. epidermidis* strains using latex agglutination and PCR methods. When compared with community strains (32%), 68% of hospital-associated CoNS strains had at least one virulence determinant in that study.[44] The production of exoenzymes and extracellular enzymes enables disruption of host tissues to stifle the immune system, thus helping the organism to establish infection. For example, elastase produced by *S. epidermidis*, damages fibronectin, albumin, and fibrinogen.[45]

Specifically, identification of true bacteremia from contamination is complicated, as there is no single benchmark with adequate level of specificity. Certain parameters have been used such as the number of positive blood cultures for CoNS obtained, correlated with clinical symptoms.[28] Studies have linked the detection of at least two positive blood cultures with clinical bacteremia; that is, CoNS were thought to play pathogenic roles if multiple positive blood cultures were obtained within a specific period. However, it has been found that about 34% of bacteremia of nosocomial origin had only one positive blood culture.[28] Thus, the sole use of this parameter may lead to underdiagnoses of clinical bacteremia due to CoNS.

Mvalo *et al.* considered CoNS as a cause of bloodstream infections (BSIs) and not a contaminant if they were isolated from at least two blood cultures within a 48-hour period.[46] Ballot *et al.*, investigating BSIs of bacterial origin in neonates, considered CoNS as contaminants if only there were no clinical signs of illness, C-reactive protein (CRP) was normal, and there was no indwelling device.[47] Similar parameters like multiple blood cultures of same microorganism isolated from distinct sites within 7 days of initial culture, with corresponding suggestive inflammatory markers (such as increased CRP and white cell count) have been used.[48]

Other indicators for determination of true bacteremia include identifying the CoNS species involved, the biotypes, antimicrobial susceptibility, and clonality. For instance, when recovered CoNS species are identical or are highly clonally related, there could be a decreased possibility of contamination and may reflect true bacteremia.[28] Thus, increased capacity for species identification and typing is required. Advances in molecular and phenotypic techniques, including WGS and spectrometric methods, have largely contributed to an understanding of mechanisms of pathogenicity. Most commonly reported CoNS species implicated in infections in this review are *S. epidermidis*, *S. haemolyticus*, and *S. xylosus* (Tables 1 and 2 and Fig. 1). Other CoNS species recovered in the various studies reviewed herein include *S. lugdunesis*, *S. capitis*,[49] *S. saprophyticus*, *S. simulans*,[1] *S. hominis*,[22] *S. warneri*, *S. cohnii urealyticum*, and *S. sciuri*.[50] It is apparent that there is a pronounced species diversity of CoNS in Africa.

The adherence to a host or surfaces of invasive devices is a vital process deployed by staphylococci to colonize host and/or establish infection. CoNS colonize the polymer surfaces of devices by forming biofilms of multiple layers.[51] This process is vital in the pathogenesis of infections associated with invasive devices.[30] *S. epidermidis*, of the CoNS species, is most frequently isolated from biofilm-associated infections.[52] The organisms most likely gain entry during the insertion of device through the skin or mucous membranes. In biofilms, there is the agglomeration of bacterial cells enclosed in vague extracellular material containing bacterial products such as polysaccharides, teichoic acids, extracellular DNA, and proteins.[52,53]

Biofilms can form on living (such as host tissues) surfaces or nonliving (such as medical devices) surfaces. Considering that biofilm formation is the most important factor in CoNS pathogenesis and virulence,[42] it is important for studies to investigate the phenomenon in CoNS to inform clinicians of its possible role in virulence. Phenotypic methods, genotypic detection of biofilm-formation genes (by PCR), or WGS are handy in this regard.

Pathogenic conversion of CoNS, where the simultaneous possession of *coa*, *vwb*, and *clfA* genes (encoding coagulation and agglutination of vertebrate blood) by CoNS was observed to have enhanced the survival of *S. simulans* in whole blood and replication in distal organs due to enhanced ability to agglutinate in plasma.[54] Thus, the possession of these determinants has been mooted as a possible mechanism of converting otherwise commensal staphylococci into invasive pathogens.[54]

Although determining the pathogenicity of CoNS can be difficult, signs of inflammation, inserted foreign body, immunosuppression, virulence determinants, and recurring detection of the same clone are generally factors that predict a high probability of infection rather than contamination.[24]

Transmission

Within the hospital, other health care facilities, and in animals, little is known about the transmission of CoNS and their outbreak potential. Multidrug resistance in CoNS, their ability to form biofilm, increased use of antibiotics and antiseptics, and the rising use of invasive devices may contribute to the transmission in these environments.[55,56] The unsolved cases of transmission attributed to circulating clones of CoNS may be underreported as many CoNS outbreak cases are unidentified. In vulnerable populations such as the immunocompromised, their contribution to significant morbidity and mortality have been acknowledged.[30]

Occurrence, Role, and Antibiotic Susceptibility of Suspected Clinically Relevant CoNS in Humans, Within Africa, Since 2009

Bloodstream infections

In a retrospective crosssectional study conducted to investigate the epidemiology of bloodstream infections (BSIs) among pediatric oncology patients in South Africa, 49.1% of blood-positive cultures identified were Gram-positive isolates, of which CoNS represented 23.1%, even though the BSIs found were generally associated with a low-case fatality rate (Table 1).[46] Indeed, Gram-positive bacteria are responsible for up to about 70% of neonatal nosocomial infections in many hospitals, with CoNS accounting for more than half of infections represented in Fig. 2.[57,58] However, Gram-negative pathogens may be more dominant as causes of neonatal infections in low- and middle-income countries, with higher rates of antibiotic resistance.[59] Opportunistic infections are a significant contributor to morbidity and mortality in pediatric oncology. The frequent use of indwelling central vascular access devices and the recurrent hospitalizations in cancer patients present an increased risk of exposure to pathogenic CoNS, leading to increased infections, which are usually multidrug resistant.[46]

A similarly high CoNS prevalence of 19.1% was recorded as a cause of BSI in neonates in a South Africa study, with 86% of them showing methicillin resistance (Table 1).[47] The vulnerability of immune systems of neonates makes them susceptible to infections, not least among them is neonatal sepsis, an infection which upon presentation may be hard to establish and is also linked with significant morbidity and mortality.[47,57] Thus, early initiation of empirical antimicrobial therapy, on suspicion of neonatal sepsis, is critical, until the condition is ruled out or the actual cause of infection is determined. The observed susceptibility patterns of bacteria to antibiotics informs the choice of empiric antibiotic treatment. Empiric antimicrobial therapy should be tailored as pathogenic causes and sensitivities of infections vary between neonatal units. No vancomycin resistance was observed in Gram positives on susceptibility testing in this study.[47] Thus, vancomycin will be useful as empiric treatment for CoNS sepsis, including MR-CoNS in this setting.

In a neonatal intensive care unit (ICU) in South Africa, CoNS were rated third (19%), behind *Acinetobacter* (21%) and *Klebsiella* (25%) as the cause of significant BSI events (Table 1).[48] BSIs (neonatal sepsis) are a significant cause of morbidity and mortality in neonatal ICUs around the world. In low/middle-income countries, there is a paucity of data on BSI in neonatal ICUs, and the menace of BSIs is compounded by the limited human and physical resources in these areas, as compared with high-income countries, where bloodstream pathogens are well described.[60]

The occurrence of multidrug resistance in CoNS presents a peculiar problem in resource-constrained countries due to lack of access to newer generation antibiotics, which are also more costly. In low/middle-income countries, an estimated 50%–78% of neonatal BSIs are caused by Gram-negative pathogens.[61] As well, in Zambia, CoNS was second (6%) only to *Klebsiella* species (75%), as the cause of neonatal sepsis in neonatal ICU of a referral center in a study that investigated the etiology of neonatal sepsis.[62]

CoNS were found among the leading etiologies of confirmed neonatal sepsis at 12.6%, coming third after *S. aureus* (31.0%) and *Klebsiella* (23.0%), in Nigeria (Table 1), with isolates showing susceptibility toward levofloxacin (95.7%), ofloxacin (95.1%), cefotaxime (86.7%), and ceftazidime (81.3%).[63] However, against the commonly used gentamicin and cefuroxime, 56.4% susceptibility was observed. There was a high rate of susceptibility to quinolones, although this

Table 1. Suspected Diagnoses, Sources, and Study Outcomes of Coagulase-Negative Staphylococci in Humans Since 2009

Country	*Year of publication*	*Source*	*CoNS species*	*Method of identification/ laboratory detection*	*Suspected/ underlying infection*	*Study population*	*Number of samples/study outcome/prevalence*	*Reference*
Benin	2016	Urine, blood, intravascular catheter	*Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *S. hominis*, *S. saprophyticus*, *S. cohnii*, *S. sciuri*, *S. arlettae*, *S. capitis*, *S. warneri*, *S. simulans*, *S. caprae*, *S. lugdunensis* and *S. kloosii*	Culture, biochemical tests, API STAPH (speciation), MALDI-TOF Biotyper™	Bacteremia, UTIs	In- and out-patients	99 clinically relevant CNS isolates (62 hospital and 37 community acquired)	44
Democratic Republic of Congo	2014	Wound swabs	NA	Culture, latex agglutination test	Surgical site infections	Hospitalized surgical patients	15/130 (11.5%) were CoNS	6
Egypt	2017	Blood	*S. lugdunesis*, *S. haemolyticus*, *S. capitis*, *S. epidermidis*. Three unidentified	Coagulase test, API STAPH 32 for speciation, PCR (negative coagulase (*coa*) gene)	BSIs	ICU patients	58 isolates out of 743 blood samples (7.8%)	49
Ethiopia	2013	Blood	NA	Culture, biochemical tests	Septicemia	Suspected septicemia patients	42.3% of culture-positive isolates	70
Ethiopia	2014	Urine samples (midstream)	NA	Culture, biochemical tests	UTI	Pregnant women	15 (32.6%) of 46 asymptomatic bacteriuria cases	76
Ghana	2013	Blood	NA	Culture, biochemical tests	Sepsis	Infants and children up to 14 years	28.7%	69
Kenya	2017	Blood	NA	Blood culture	Neonatal BSIs	Neonates	10% of neonates had CoNS isolated from blood culture, some 43.8% of all positive cultures were CoNS	68
Morocco	2013	Blood, urine, and stool	*S. epidermidis*	Blood culture	Pneumonitis, mucositis, fever of unknown origin, meningitis	Hospitalized children and young adults (<30) with leukaemia	14/30 from blood (46.6%)	40

(*continued*)

Table 1. (Continued)

Country	*Year of publication*	*Source*	*CoNS species*	*Method of identification/ laboratory detection*	*Suspected/ underlying infection*	*Study population*	*Number of samples/study outcome/prevalence*	*Reference*
Nigeria	2009	Blood, CSF	NA	Culture, coagulase test	Acute systemic infections (meningitis)	Children (2–60 months)	1/202 (0.5%)	85
Nigeria	2010	Blood	NA	Culture, biochemical tests	Sepsis	Neonates	12.6%	63
Nigeria	2010	Blood	*S. epidermidis*, *S. warneri*, *S. cohnii* subspecies *urealyticum*, *S. cohnii cohnii*, *S. chromogenes*, *S. sciuri*, and *Staphylococcus xylosus*	Culture, standard biochemical tests, API 20E and API Staph Kits	BSI	HIV-infected adults	15 out of 26 (58%) bacterial isolates	50
Nigeria	2013	Venous blood samples	NA	Culture, gram staining, catalase test, coagulase test	Bacteremia	Patients undergoing cleft lip and palate surgery	14/40 (35%) of all positive blood samples	64
Nigeria	2013	Cerebrospinal fluid, blood	NA	Culture	VP shunt infections	Pediatric hydrocephalic patients	29.4%	72
Nigeria	2013	Urine, wound, urinary catheter, blood, eye infections, exudates and swabs from genital infection etc	*S. haemolyticus*, *S. epidermidis* and *S. saprophyticus*, *S. chromogenes*, *S. xylosus*, *S. simulans*	Culture, Microbact Staph ID system (Oxoid product) for CoNS speciation, coagulase test (slide and tube)	Septicemia, otitis media, tonsillitis, and pharyngitis	Hospital patients	79 selected CoNS isolates	1
South Africa	2018	Blood	NA	BACTEC 9240 automated blood culture system, BacT/ALERT automated blood culture system biochemical tests	BSIs	Pediatric oncology patients	40/173 (23.1%)	46

(continued)

Table 1. (Continued)

Country	Year of publication	Source	CoNS species	Method of identification/ laboratory detection	Suspected/ underlying infection	Study population	Number of samples/study outcome/prevalence	Reference
South Africa	2010	Burn wounds	NA	Culture and biochemical tests	Burn wound infections	Adults and pediatrics	20/629 (3.2%)	82
South Africa	2012	Blood	NA	Culture	BSIs	Neonates	19.1% (47/246), 7% of CoNS-related cases led to death	47
South Africa	2014	Blood	NA	Culture	BSIs	Neonates	22/78 (28%), with 11 responsible for significant BSI	48
South Africa	2018	Intravascular catheters, blood	*S. epidermidis*	BacT/ALERT 3D system, VITEK® 2 automated system, MALDI-TOF MS, PCR	CRBSI	Adult and pediatric patients	*S. epidermidis* cause of 31% of CRBSI	75
Tanzania	2010	Blood	NA	Culture, biochemical tests	BSIs	Hospital patients	67.4% of culture-positive samples	71
Tunisia	2011	Blood, pus, catheter, graft tissue	*S. haemolyticus*, *S. hominis*	Culture and biochemical tests, API ID32 STAPH	Not specified	Neutropenic patients	142 CoNS strains	22
Zambia	2016	Blood	NA	Culture, biochemical testing (API) etc	Neonatal sepsis	Neonates	6%	62
Zimbabwe	2015	Urine	NA	Culture and biochemical (coagulase, catalase) tests	UTIs	Symptomatic hospital patients	126/754 urine samples (16.7%)	12

BSI, bloodstream infections; CoNS, coagulase-negative staphylococci; CRBSI, catheter-related bloodstream infection; CSF, cerebrospinal fluid; ICU, intensive care unit; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NA, not available (species identification not done); PCR, polymerase chain reaction; UTI, urinary tract infections; VP, ventriculoperitoneal.

TABLE 2. SUSPECTED DIAGNOSES, SOURCES, AND STUDY OUTCOMES OF COAGULASE-NEGATIVE STAPHYLOCOCCI IN ANIMALS SINCE 2009

Country	*Year of publication*	*Source*	*CoNS species*	*Method of identification/lab detection*	*Suspected/underlying infection*	*Study population*	*Number of samples/study outcome/prevalence*	*Reference*
Algeria	2011	Milk	*S. xylosus*, *S. epidermidis*, *S. hominis*, *S. lentus*	Culture, biochemical tests	Subclinical mastitis	Cows with subclinical mastitis	23/89 bacterial strains (25.8%)	117
Ethiopia	2019	Milk	*S. epidermidis*	Culture, OMNILOG/BIOLOG automated bacterial identification system	Mastitis	Dairy cattle	10.5%	109
Rwanda	2019	Milk	*S. epidermidis*, *S. sciuri*, *S. chromogenes*, *S. xylosus*, *S. haemolyticus*, *S. capitis*	Culture, MALDI-TOF MS	Mastitis	Dairy cattle	40.2%	108
South Africa	2009	Milk	NA	Culture, biochemical tests	Mastitis, teat canal infections	Lactating and non-lactating cows	61.71%	96
South Africa	2015	Milk, human nasal swabs	*S. chromogenes*, *S. epidermidis*	Multiplex PCR, MALDI-TOF MS, *tuf* gene sequencing	Bovine mastitis	Cattle and close human contacts	102/3,387 milk samples (3.0%)	26
South Africa	2017	Milk	*S. xylosus*, *S. chromogenes*, *S. hominis*, *S. warneri*, *S. sciuri*, *S. epidermidis* and *S. saprophyticus*	Culture, API Staph test kits	Mastitis	Dairy cattle	66.3%	98
South Africa	2018	Milk	NA	Culture	Mastitis	Cattle	29.60% (total mixed ration-based dairies), 26.90% (pasture-based dairies) in 2008; 20.20% and 22.70% respectively for 2013	118
Tunisia	2018	Milk	*S. xylosus*, *S. warneri*, *S. chromogenes*, *S. saprophyticus*, *S. haemolyticus*, *S. sciuri*, *S. equorum*, *S. pasteuri*, *S. cohnii*	Culture, biochemical tests, PCR	Clinical mastitis	Cattle	68/300 samples CNS species (22.7%)	41

(*continued*)

TABLE 2. (CONTINUED)

Country	*Year of publication*	*Source*	*CoNS species*	*Method of identification/lab detection*	*Suspected/underlying infection*	*Study population*	*Number of samples/study outcome/prevalence*	*Reference*
Tunisia	2019	Milk, manure and human nares	*S. equorum, S. xylosus, S. sciuri, S. saprophyticus, S. haemolyticus, S. lentus, S. vitulinus and S. cohnii*	API Staph, 16S rRNA followed by sequencing	Mastitis	Cattle	40/127	105
Uganda	2013	Milk, nasal samples	*S. saprophyticus, S. xylosus, S. sciuri, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. gallinarum, S. pasteuri*	Culture, tube coagulase test, DNase test, Phoenix Automated Microbiology System' (Phoenix 100 ID/AST system) for species identification	Mastitis	Milkmen and mastitis-infected cattle	20 out of 82 bacterial isolates (24.3%)	97
Uganda	2014	Quarter milk samples	*S. epidermidis, S. haemolyticus*	Culture, coagulase test, MALDI-TOF	Subclinical mastitis	Dairy cattle	31.7% of 116 samples	100
Zimbabwe	2013	Milk	NA	Culture and biochemical tests	Mastitis	Dairy cattle	27.6% CoNS samples	107

NA, not available (species identification not done).

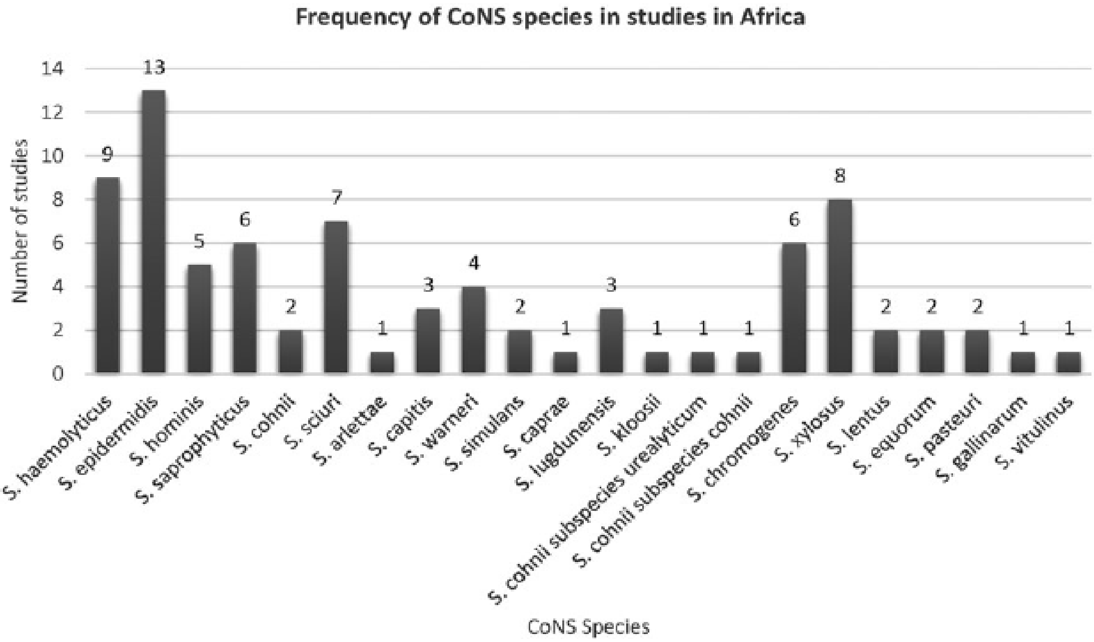

FIG. 1. A graph showing the frequency of CoNS species according to the number of studies in the last decade. CoNS, coagulase-negative staphylococci.

class is reportedly unsafe for newborns. Prompt empirical treatment based on likely etiology and observed susceptibility patterns is useful, as results from laboratory investigation may not be available between 48 hours and 7 days.

The reduced susceptibilities toward commonly used antibiotics such as cefuroxime (37.5%), ampicillin (37.5%), cloxacillin (15.4%), and amoxicillin/clavulanic acid (47.4%) in that study setting, is worrying as the focus of clinicians will be shifted to deploy newer generation antibiotics, with increased cost of treatment. Furthermore, the increased use of newer generation or last-resort antibiotics selects for the development of resistance, which becomes problematic especially when bacteria disseminate ARGs to hitherto susceptible bacteria. Patients with poor perinatal events need to be monitored carefully and treated until sepsis is ruled out especially those with high risk factors such as low birth weight, low socioeconomic status, protracted labor, and prolonged rupture of membranes.[63]

CoNS was implicated in 35% of positive blood samples as a cause of bacteremia in patients undergoing cleft lip and palate surgery (Table 1).[64] As *Staphylococcus* species are usual colonizers of the skin and nasal passages, incisions made during cleft repair surgery cause the otherwise commensals to encounter mucous membranes of skin and nasal passages. Thus, *Staphylococcus* species, and particularly CoNS in this study, may enter the bloodstream through contaminated cleft lip and palate wounds.[64] Even though CoNS were the most commonly isolated pathogens, cases of bacteremia associated with cleft lip and palate surgery were polymicrobial in nature, due to the polymicrobial nature of the oral cavity, necessitating the use of broad-spectrum antibiotics for prophylaxis and treatment in vulnerable groups.[64]

Among HIV-infected adults consecutively attending HIV clinic at a tertiary-level teaching hospital in Nigeria, a CoNS prevalence of 58%, ahead of Gram-negative nontyphoid *Salmonella* spp (23%) and *Salmonella typhimurium* (15%), was found as cause of bacterial BSIs.[50] Antimicrobial susceptibility testing showed that CoNS isolates displayed high or complete susceptibility to ceftazidime (66%), cefotaxime (66%), amoxicillin/clavulanic acid (100%), cefuroxime (73%), piperacillin/tazobactam (100%), oxacillin (100%), and vancomycin (100%), but mainly showed resistance against ampicillin (73%), tetracycline (53%), and penicillin (73%). BSIs have been frequently associated with HIV/AIDS patients and are estimated to account for significant morbidity and mortality within this population.[50] Considering that HIV/AIDS patients have compromised immune systems, the presence of CoNS as a leading cause of BSIs is unsurprising as it has been established that CoNS species are a leading cause of infection in immunocompromised patients.[65]

Vancomycin heteroresistance in CoNS BSIs in ICU patients was investigated in a study that found a CoNS prevalence of 7.8% as probable cause of BSIs, of which 75.9% were oxacillin resistant.[49] Genotypic detection by PCR found all oxacillin-resistant isolates contained the *mecA* gene. Vancomycin heteroresistance in CoNS isolates was detected by population analysis profile even though all CoNS isolates were susceptible to vancomycin. This observation presents a growing health concern. Thus, stricter screening methods are recommended to detect them and improve treatment outcomes of patients. CoNS are major culprits in the bacterial etiology of BSIs in ICU patients.[66] Because of the increasing resistance toward β-lactam antibiotics in CoNS, clinicians rely on glycopeptides (particularly vancomycin) as first-line antibiotics in the treatment of severe infections caused by CoNS.[67]

Whereas CoNS are considered as major neonatal pathogens in upper and high-income countries, little is known about their epidemiology, role, and coverage for empiric anti-Staphylococcal treatments in Africa.[68]

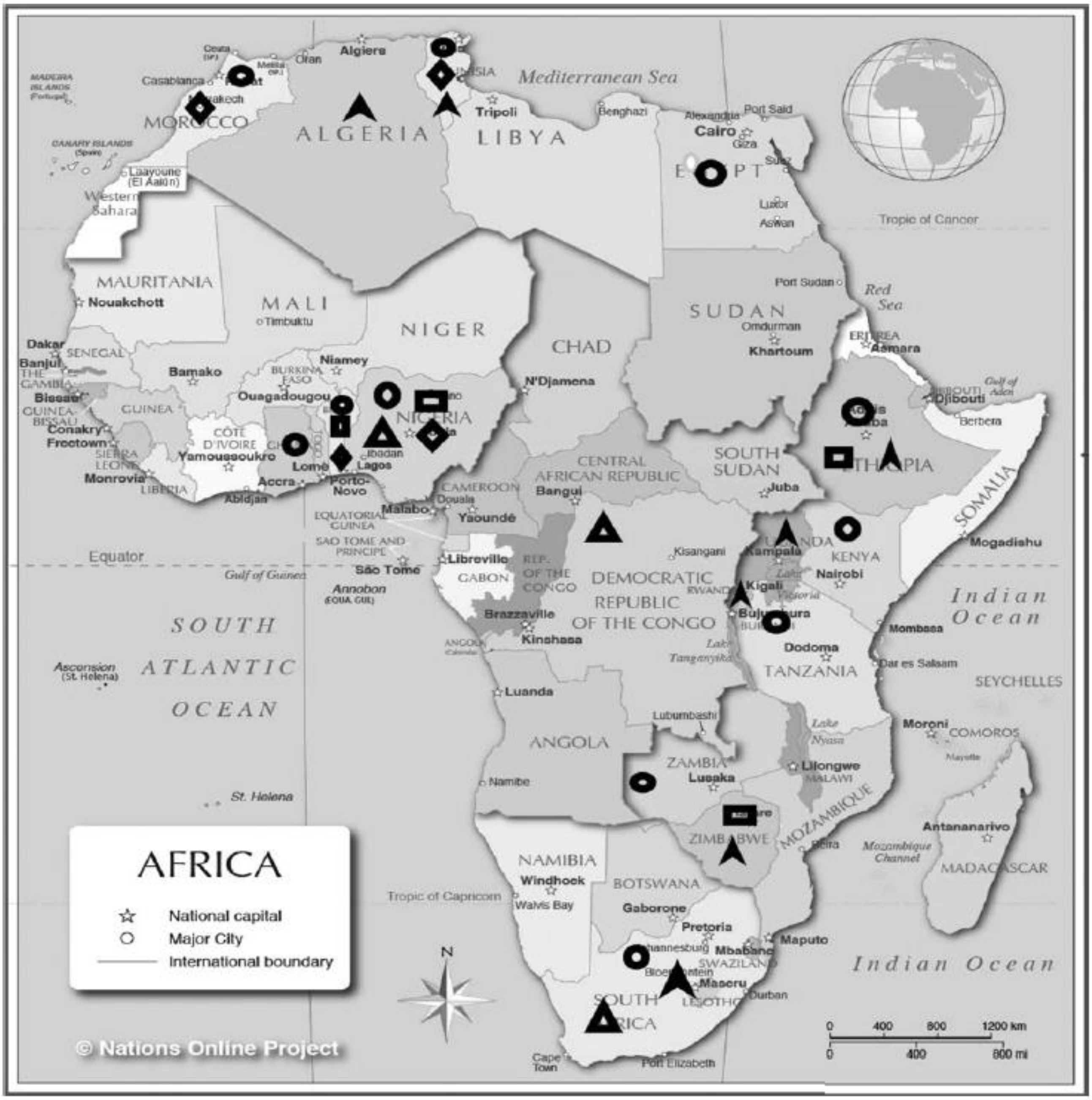

FIG. 2. Geographic distribution of CoNS infections in Africa between 2009 and 2019 (Map was reproduced from Nations Online Project). Map of Africa showing countries where CoNS were reported from 2009 to 2019. Most represented countries were Nigeria and South Africa. ⭘, bloodstream infections; ☐, urinary tract infections; △, wound infections; ◇, multiple source infections; ⋀, intramammary infections (cattle).

Seale *et al.* conducted a study to ascertain the prolonged duration of admission and case fatality rate of CoNS-associated neonatal infections in Kenya and observed no difference in case fatality rates between CoNS-associated infections and other infections (Table 1).[68] Again, it was observed that CoNS did not lead to prolonged duration of hospitalization. Treatment protocol used by the hospital under study for neonates was in line with WHO guidelines, namely ampicillin and gentamicin as first line, and a third-generation cephalosporin as second line. There was no specific anti-Staphylococcal agent in the empirical treatment, although gentamicin may provide cover for staphylococcal isolates sensitive to methicillin.[68]

There may not be a distinct need to target staphylococci in empirical treatment in neonates, any more than would be considered for Gram-positive pathogens. Of critical importance, however, is to pay attention to the observed susceptibility patterns of antibiotics used empirically, while tailoring the need for changes. CoNS are more significant as a cause of infection after admission, and their role in infection may be understated depending on the time of sampling; late stage sampling may increase the significance of CoNS, which hitherto had been commensals.[68] More invasive support techniques in neonatal practice may increase the significance of CoNS as important causative pathogens in resource-constrained countries.

Varying incidence of 28.7%,[69] 42.3%,[70] 67.4%,[71] and susceptibilities of CoNS in septicemia have been recorded in Ghana, Ethiopia, and Tanzania, respectively (Table 1). It is observable that there is a wide range of incidence of CoNS in BSIs from studies across Africa. The varying incidence could be due to geographical factors, sampling techniques, different laboratory detection capacities, among others.

Invasive device-related infections

CoNS were the most common etiology (29.4%), ahead of *S. aureus* (23.5%) and *Escherichia coli* (11.8%) in a study conducted to investigate the contributing factors and outcomes of shunt infection in Nigeria (Table 1).[72] Shunt infection was defined in the study as positive cerebrospinal fluid (CSF) with positive shunt component culture. Ventriculoperitoneal (VP) shunt is the main surgical procedure used in the treatment of hydrocephalus. However, the increase in VP-associated infections has caused significant morbidity and mortality in VP-treated hydrocephalic children, with the skin mostly the source of infection.[72] The early detection and swift initiation of therapy are critical in the success of treatment of infections associated with VP shunts. The findings of the study agreed with general literature that CoNS and *S. aureus* are the most commonly reported causes of shunt infections.[73,74] The formation of mucoid biofilms by CoNS favors their adherence to shunt hardware, thus enhancing their survival. Of significant note was that the mortality rate in patients with shunt infection was 21.4% as compared with 2.7% in those without shunt infection.[72] Treatment of CoNS in invasive device-related infections has been discussed under "treatment" section.

Bouchami *et al.* found that 31% of the total CoNS isolates were from catheters,[22] whereas foreign body-related infection accounted for 1% of CoNS infections in a Benin study.[44]

Ehlers *et al.* while investigating catheter-related bloodstream infections (CRBSI) in a South African hospital, determined *S. epidermidis* as the cause of infection in 31% of CRBSI with isolates displaying complete resistance to β-lactams and high resistance to gentamicin (81%) and erythromycin (86%). The study also showed that 81% of *S. epidermidis* isolates carried the *qac*A/B and *ica*AB genes each.[75]

Urinary tract infections

CoNS have emerged as a principal cause of UTIs especially among immunocompromised and hospitalized patients with the high occurrence of hospital-acquired UTIs attributed to the use of urethral catheters.[5]

The occurrence of asymptomatic bacteriuria and antibiotic susceptibility in pregnant women seeking antenatal care in an Ethiopian teaching hospital was studied, and found CoNS to be responsible for 32.6% of asymptomatic bacteriuria cases, compared with 26.1% for *E. coli* and 13% for *S. aureus* (Table 1).[76] Infections were mostly polymicrobial, with the highest proportion of polymicrobial infection recorded for CoNS and *E. coli* at 8.7%. Previous studies about asymptomatic bacteriuria in pregnant women in Ethiopia have reported incidences of 7%–10.6%, with *E. coli*, *S. aureus*, *Klebsiella* species, and *S. saprophyticus* frequently implicated.[77,78] Thus, the incidence of asymptomatic bacteriuria in pregnant women in Ethiopia may be rising and steps need to be taken to reduce the risk of complications and perinatal events. Screening for asymptomatic bacteriuria, particularly commonly implicated bacteria, like CoNS, will enable early detection and forestall the development of complications.

The antibiotic susceptibility patterns and virulence factors of CoNS bacteria associated with UTIs was studied in Zimbabwe, where high resistance rates were found against oxacillin (69.8%), cotrimoxazole (72.2%), and nalidixic acid (88.1%), while showing high susceptibilities toward gentamicin (68.3%) and nitrofurantoin (79.4%).[12] The *mecA* gene (methicillin resistance) was detected in 62.5% of the CoNS isolates. The *ica* AB genes (encoding biofilm formation) and *atl* E (encoding the vitronectin-binding cell surface protein which facilitates primary attachment) were detected in 32.5% and 25% of isolates, respectively. The *ica* gene has been found as the most commonly detected virulence gene in CoNS highlighting the importance of biofilm formation in the virulence of pathogenic CoNS.[12]

The multidrug resistance profile of biofilm producers was underscored by the observation that 9 out of the 13 *ica* AB gene-positive isolates (which also possessed the *mecA* gene) were resistant to most of the antibiotics tested in the study, although no direct relationship between *ica* AB gene detection and resistance was established. What was apparent though, was the association between biofilm formation and multidrug resistance. Furthermore, the detection of *ica* AB and *mecA* genes, may be an important determinant to differentiate between infectious and innocuous strains of CoNS.[12]

S. haemolyticus (29%), *S. saprophyticus* (25%), and *S. epidermidis* (13%) were the main causes of CoNS-associated UTIs in community and hospital infections in Benin.[44] It is known that *S. saprophyticus* produces urease, which is essential for colonization and protection in host tissues, by causing tissue damage and invasion and is an important marker of bacterial infections.[79,80] Specifically, *S. saprophyticus* subsp. *saprophyticus* is known for its preference for the rectum and genitourinary tract particularly in young women; this genitourinary adaptation is supported by their increased affinity for hemagglutination and adhesion to uroepithelial cell fibronectin.[39,81] It is also the second most reported cause of uncomplicated lower UTIs in young females who are sexually active. These factors make the isolation of CoNS especially *S. saprophyticus*, in the genitourinary tract significant.[30,39] It is therefore important to determine the specific CoNS species to confirm relevance in UTIs. Thus, the lack of species determination in some studies investigating CoNS in UTIs is a shortfall.

Wound infections

The colonization of burn wounds by microorganisms is a common occurrence especially with major burn wounds. Initially, organisms residing in the skin and throat, such as *Staphylococci* and *Streptococci*, may colonize wounds and may be followed later by gastrointestinal bacteria such as *E. coli*, *Proteus*, and *Klebsiella*.[82] Also, formites and hands of personnel can serve as sources of bacteria in the transmission of infections in burn wounds. These pathogens can cause overt wound infection and can lead to bloodstream invasion, leading to delayed wound healing and sepsis-related mortality.[82] In a South African study to characterize burn wounds, CoNS were less prevalent (3.2%) compared with *S. aureus* (27.7%), *Klebsiella pneumoniae* (13.4%), and *Proteus mirabilis* (12.4%).[82]

Conversely, CoNS accounted for a high incidence of surgical site infections, following surgical procedures, when surgical site infections were characterized (Table 1).[6] Surgical site infections are infections affecting either the incision or deep tissue at the site of operation, and occurs up to 30 days postsurgery or up to 1 year in cases of those with implants.[6] Although improvements have been made in the prevention of postsurgical infections, surgical site infections remain problematic in surgical practice and account for increased cost of health care and prolonged hospitalization.[83] Studies show that most of the causes of surgical site infections are normal residents of the skin, such as CoNS and *S. aureus*.[83,84] The MR-CoNS detected were 0% resistant to vancomycin. Increased resistance of CoNS in such instances may be due to increased use of broad-spectrum antibiotics or prolonged hospital stay.

Multiple-source infections

Blood and CSF were analyzed to investigate severe infections due to invasive bacteria in a Nigerian hospital and detected a CoNS prevalence of 0.5%. CoNS were resistant to cotrimoxazole and erythromycin with a suspected diagnosis of meningitis.[85] The impact may be underestimated due to lack of diagnostic capacity in resource-constrained countries, as many diseases are not properly investigated.

Azih and Enabulele demonstrated that 34.2% of the CoNS isolates from various sources demonstrated hemolysis on blood agar, with *S. haemolyticus* (58.8%) and *S. epidermidis* (25%) showing the most hemolytic activity. Again, 75.95% of CoNS samples demonstrated slime production, with all *S. simulans* and *S. xylosus* isolates showing slime production.[1] *S. haemolyticus* and *S. saprophyticus* were isolated from infection sites with suspected cervicitis and pelvic inflammatory disease. *S. epidermidis*, *S. saprophyticus*, and *S. simulans* were implicated in ear, nose, and throat infection in that study.[1]

CoNS were the commonest bacteria isolated in a study conducted to describe the clinical features and microbiological characteristics of febrile illnesses in in-patients being treated for acute lymphoblastic leukemia and acute myeloid leukemia.[40] Febrile neutropenia and other kinds of infections are common in patients with acute leukemia, leading to decreased survival rates, with studies attributing 60% of deaths in acute lymphoblastic leukemia patients to complications of infectious diseases.[86] Delays in initiating antibiotic therapy and hospitalization can be contributing factors to increased mortality.

While investigating the antibiotic susceptibility of CoNS in a Tunisian study, Bouchami *et al.*, as expected, found MR-CoNS to be more resistant to antibiotics compared with methicillin-susceptible CoNS, but the MR-CoNS were susceptible to vancomycin, and displayed only 6% resistance to teicoplanin.[22] Methicillin resistance in pathogenic CoNS further complicates antibiotic therapy and restricts treatment options, with some studies reporting oxacillin resistance in CoNS approaching 90%.[22,87,88] The complete susceptibility of isolates in that study to vancomycin is significant as the antibiotic is used in neutropenic patients as empiric therapy. The first case of teicoplanin and vancomycin resistance in CoNS was reported in *S. haemolyticus*. This species is thus considered particularly inclined to develop glycopeptide resistance among the CoNS group.[89]

Nanoukon *et al.* found high resistance rates of CoNS to various antibiotics, including penicillin (92%), fosfomycin (81%), cefoxitin (74%), and trimethoprim/sulfamethoxazole (72%). That most of the virulence determinants such as esterase, enterotoxins, protease, and hemolysins were from blood isolates in that study, may suggest that these CoNS isolates may be adapted to colonize blood.[44] The most frequently isolated CoNS species in that study were *S. haemolyticus* (44%), *S. epidermidis* (22%), and *S. hominis* (7%).

Comparatively, regarding the transmission in health care facilities and their ability to cause outbreaks globally, little is known about CoNS compared with *S. aureus* and many CoNS outbreak cases globally remain unidentified. However, a number of antibiotic-resistant clones of CoNS have been found as causes of outbreaks in Italy[90] and Brazil.[91] As well, CoNS have been implicated in BSIs,[92] CRBSI,[93] and continuous ambulatory peritoneal dialysis-related infection peritonitis[94] in Turkey, Germany, and South Korea, respectively at varying levels of prevalence.

Occurrence, Role, and Antibiotic Susceptibility of Suspected Clinically Relevant CoNS in Animals, Within Africa, Since 2009

Foodborne diseases contribute to significant morbidity and mortality worldwide and cause great economic losses. The sporadic or outbreak cases of foodborne diseases worldwide coupled with the globalization of food supply results in food safety and security concerns. An understanding of the causative pathogen will inform appropriate food safety and public health policies.[95]

CoNS are frequently isolated pathogens in herd milk samples. There has been a shift in mastitis-associated pathogens over the years with hitherto less common pathogens becoming more frequently involved in mastitis pathogenesis.[96] For instance, *Streptococcus agalactiae* was the major mastitis pathogen before the use of penicillin G in 1943, but *S. aureus* has since replaced *S. agalactiae* as the major mastitogenic pathogen.[96]

Mastitis is an inflammation of the mammary gland usually caused by microorganisms that colonize the udder. It can result in severe economic losses as a result of decreased milk yield, poor quality of milk, and high cost of treatment in affected cows and affects the dairy industry worldwide.[97] CoNS are leading etiological agents in mastitis worldwide, however in Africa, little is known about their involvement and antibiotic susceptibility, including methicillin resistance. Bacterial pathogens continue to increase in relevance due to factors such as the development of newer antibiotics, increased milk production, housing systems, and milk-making machines.[96]

Milk samples from dairy herds in South Africa were examined to specifically observe the trends of microorganisms under field conditions.[96] There was an observed decrease in mastitis over the period considered (1996–2007). The most frequently isolated microorganisms from milk samples were CoNS, in both dry (61.71%) and lactating cows (60.96%) (Table 2). This was followed by *S. aureus* (17.28% for dry and 16.9% for lactating cows).[96] Due to its ability to establish chronic infection and its economic impact, *S. aureus* remained the major mastitogenic pathogen in South Africa, but CoNS was found to be the cause of mastitis in majority of cases, underlining the increasing importance of CoNS in livestock. CoNS was responsible for 71.5% of teat canal infection in quarter milk samples from 1996 to 2007.

Furthermore, pertaining to South Africa, a study found that increase in herd size, poor milking practices, and poor maintenance of milking machines increased udder exposure to pathogens.[96] CoNS need to be looked at in a new light considering that most mastitis cases in South Africa were caused by CoNS. The study was conducted in seven out of nine provinces in South Africa, thus it reflects the general state of udder health of cattle in the country even though varying factors such as management skills, milking frequency, milk yield and herd size may limit the generalization of results. Despite the development and improvement in animal husbandry practices, bovine mastitis remains a cause for economic concern due to the economic losses the disease carries.[96] It is thus important to be acquainted with possible mastitogenic pathogens, and their epidemiology, trends in disease causation to help in effective infection control and policy regulations.

A similar CoNS prevelance rate of 66.3% was observed in another South African study, which aimed to determine the distribution of mastitogenic pathogens.[98]

In another study, multidrug resistance was observed in 37.3% of isolates, among the CoNS of bovine origin, whereas 89.5% of CoNS from close human contacts were multidrug resistant (Table 2).[26] The high level of multidrug resistance in human CoNS suggests humans could be reservoirs for resistance genes. In bovine CoNS, complete susceptibility was observed toward cefoxitin, cephalothin, moxifloxacin, clindamycin, erythromycin, gentamicin, and linezolid, whereas the highest resistance values were observed against penicillin (37.3%) and ampicillin (36.3%). These two β-lactam antibiotics are commonly used in the prophylaxis and treatment of IMIs in dairy cows, being found in more than half of preparations used for such purpose in South Africa. The different susceptibility patterns observed across the continent may be explained by different antibiotic usage as well as difference in implementation of antibiotic regulation. Pathogenic CoNS causes of IMIs may be herd specific and linked to certain management practices. *S. chromogenes* has been found to be specifically adjusted to udder, making it a persistent cause of IMIs.[99]

MR-CoNS were prevalent in 57% of cows in a study, which also recorded vancomycin-resistant CoNS (*S. hominis* and *S. lugdunensis*).[97] Even though similar species were seen from both bovine and human samples, different genotyping characteristics suggests there was no zoonotic transfer. All staphylococcal isolates from cows were sensitive to daptomycin, ciprofloxacin, gentamicin, linezolid, mupirocin, and moxifloxacin.

Conversely, in an Uganda study, all cattle staphylococcal isolates showed 100% resistance to penicillin G and ampicillin and were β-lactamase producers, with β-lactamase production also being observed in 80% of CoNS isolates.[100] Intramammary infusions of either tetracycline or ampicillin were commonly used in mastitis cases, and this could partly be responsible for resistance observed against tetracycline (33%). Improved milking practices such as use of milking machines instead of hand milking and improved udder hygiene are important. Control of mastitis requires effective husbandry practices. The heavy use of antibiotics in livestock production for treatment and as prophylaxis, has been in part, blamed for contributing to antimicrobial resistance.

Klibi *et al.* while characterizing MR-CoNS and methicillin-susceptible coagulase-negative staphylococci (MSCoNS) from cows with clinical mastitis, found that 70% of MR-CoNS carried the *mecA* gene, but all MR-CoNS isolates lacked the *mecC* gene; however, the resistance genes *tet*(K), *dfr*(A), *lnu*(B), *erm*(C), *erm*(B), *erm*(T), *mph*(C), or *msr*(A) were detected.[41] The phenotypic observance of methicillin resistance without the genotypic detection of *mecA* or other known methicillin resistance mechanisms may be due to the emergence of a new methicillinase, overproduction of β-lactamases, or the changes in binding affinity of penicillin-binding proteins.[101,102] It is thus important to investigate alternative methicillin resistance mechanisms.

The variety of ARGs in CoNS in milk of mastitis cows could lead to horizontal transfer to other staphylococcal species, some of which are more virulent, leading to food safety concerns. Methicillin-resistant *S. epidermidis* for example may play the role of reservoirs of ARGs which can be transferred. This occurrence is more of a concern considering *S. epidermidis* is a suspected zoonotic pathogen. In comparison to MR-CoNS, the MS-CoNS isolates showed lower levels of resistance to antibiotics.[41] This was expected, as methicillin resistance is known to cause cross resistance to other antibiotics.[103] Dhaouadi *et al.* however, reported the first occurrence of *mecC* gene in CoNS (*S. sciuri*) in Africa (having been already reported in animals in Europe),[104] obtained from cows and manure, in a study which also found the ARGs *tet*(K), *mec*A, *msr*(A), *blaZ*, *erm*(A), and *erm*(B) simultaneously from cow (milk), manure, and human (nares) sources, which may be suggestive of an exchange of ARGs among these sources.[105] In the study by Dhaouadi *et al.*, *mec*A was predominantly found in *S. sciuri*, which was also the only species to possess the *mec*C gene, stressing its importance as reservoir. Furthermore, the possession of *mec*A and *mec*C by *S. sciuri* in cow milk and manure represents a threat of transfer to humans, especially as *S. sciuri* has been reported as a cause of severe human infection.[106] Other CoNS prevalence studies in mastitis have found 27.6%,[107] 40.2%,[108] and 10.5%[109] in Zimbabwe, Rwanda, and Ethiopia, respectively, as causes of mastitis.

In comparison to the global situation, the increasing importance of CoNS in IMIs is evinced by their high occurrence in countries such as Belgium,[110] Germany (9.1%),[111] Sweden (16%),[112] and the United States (15%).[113]

Typing methods for CoNS

Clonal diversity/relatedness studies in CoNS have not been given much attention as compared with *S. aureus*. Pulsed-field gel electrophoresis (PFGE) has shown that CoNS species, such as *S. lugdunensis, S. haemolyticus, and S. schleiferi* are not as clonally diverse as *S. aureus*, which is characterized by a wide genomic diversity.[114,115] *SCC*mec typing is important for characterizing MR-CoNS clones in epidemiological studies.

While investigating the characterization of methicillin-resistant and susceptible CoNS obtained from cow milk samples, Klibi *et al.*, using *SCC*mec typing, discovered methicillin-resistant *S. epidermidis* possessed *SCC*mec type-Iva.[41] The isolates also possessed the recombinase genes *ccr* A2/B2-class B *mec* gene complex determined by PCR of the *ccr* recombinases (1–5). Also, Ehlers *et al.* found that *SCC*mec type IV was the most predominant with 31% of *S. epidermidis* isolates belonging to that class. The same study using MLST, grouped isolates mainly into the groups; sequence type (ST) 2 as the most predominant, followed by ST54, ST59, and ST490 in descending order of predominance, with one isolate being assigned to a new group, ST596.[75]

*SCC*mec types I, II, IV, and V/VII have also been reported in a Tunisian study, which also found diverse PFGE patterns within *mec*A-positive CoNS isolates. The observation of similar *SCC*mec and PFGE patterns of MR-CoNS across different sources in that study means there was the transfer and circulation of the same clones at the human/animal/environment interface.[105]

That genetic typing of CoNS has not been given attention as *S. aureus* and other pathogens is shown by the fact only a few studies performed genetic typing even when CoNS were the major pathogens suspected of causing infection. Thus, it is difficult to ascertain the most common clones or types circulating on the continent. The aforementioned methods together have served microbiologists well, however, they are limited in their ability to discriminate bacterial strains that originated from a single bacterial clone.[116] Thus, WGS is an ideal epidemiological typing tool due to its ability to detect single changes in the genomes between two isolates and can identify new types or subtypes.[34] Effective epidemiological typing can assist in, for example linking hospital-endemic clones of CoNS to possession of resistance and virulence determinants.[42] Although high-throughput sequencing techniques are relatively cheaper now, in resource-constrained settings, such as in most parts of Africa, it has yet to be a frequent tool employed in routine epidemiological studies.

Treatment of CoNS infections

Treatment options for CoNS are limited because many of them are methicillin resistant. Thus, the glycopeptides (particularly vancomycin) are relied upon, especially in infections caused by the *S. epidermidis* group.[30] For isolates that are sensitive to methicillin, penicillin, and first- or second-generation cephalosporins may be used. On the other hand, cotrimoxazole or newer generation antibiotics such as linezolid, daptomycin may be administered, where methicillin resistance is observed.[30] Infections associated with invasive devices should have tailored treatment regimens, considering the role of the devices. These include catheter-associated UTIs. In these cases, removal of devices may be considered, and comes with additional medical procedures with associated extra costs. In some instances, removal of device can be enough to curtail infection. If devices are retained, antimicrobial therapy should be given 10–14 days.[30] Susceptibility patterns of CoNS should be considered to inform both empiric and specific treatment.

Methods & Results Obtained

Literature search

PubMed, Google Scholar, Science Direct and Web of Science were searched for English research articles, using the following combined search term: ''(Coagulase-negative staphylococcus*) AND (Africa OR West Africa OR East Africa OR Southern Africa OR North Africa OR Central Africa)''.

Study selection

Articles published in 2009 or later (from 2009 to 2019), describing clinically relevant CoNS, including patterns, frequency, epidemiology, diagnoses, and treatment in Africa were included. Non-English articles were excluded. Only research articles, wherein CoNS were suspected to have caused infections in both humans and animals, were included. All articles that did not discuss CoNS, those not conducted in Africa and those published before 2009 were excluded. All abstracts, reviews, and conference proceedings or articles were not included. The search yielded >1,700 items. Of these, 35 articles were included based on the inclusion criteria described above.

Limitations, future perspective, and conclusions

The increasing availability of next-generation sequencing tools due to their decreasing costs has yet to be reflected in Africa, by their scarce use in routine investigations, at least in large microbiology laboratories. The acquisition of bench-top high-throughput sequencing devices will be important to investigate outbreaks of CoNS infections at reduced turnaround times. This will lead to a thorough assessment of the true infectious/virulent potential of isolated CoNS species and inform prompt clinical decisions.

CoNS are becoming increasingly relevant, clinically and epidemiologically, as seen in the number of infections, morbidity, and mortality attributed to them. Although they are mostly classified as mere contaminants, they may be the true causes of infections in certain cases. There is a marked species diversity of CoNS across the continent of Africa, and there is the need for increased laboratory capacity for effective speciation of CoNS at reduced costs. The deployment of molecular and spectrometric techniques for detection where affordable are needed to complement basic biochemical methods for effective diagnoses.

Care should be taken in patients harboring invasive devices, while decreasing the risk for contamination during insertion and sample taking. The lack of epidemiological typing of CoNS, lack of virulence screening, and the lack of assessment of the immune status of subjects in many studies considered, limit the assessment of the true pathogenic potential of CoNS species in Africa. Further studies investigating the role of CoNS, including thorough characterization of recovered need, are to be carried out in Africa.

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Address correspondence to:
Jonathan Asante, BPharm, MPharm
Antimicrobial Research Unit
College of Health Sciences
University of KwaZulu Natal
Private Bag X54001
Durban 4000
South Africa

E-mail: josante33@yahoo.com

Chapter 3 – Manuscript 2

MULTIDRUG-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI FROM THE UMGUNGUNDLOVU DISTRICT OF KWAZULU-NATAL PROVINCE IN SOUTH AFRICA: EMERGING PATHOGENS.

AUTHOR CONTRIBUTIONS

- **Jonathan Asante**, as the principal investigator, co-conceptualized the study, undertook the laboratory work and drafted the manuscript.
- Bakoena A. Hetsa, aided with analysis and undertook critical revision of the manuscript.
- Daniel G. Amoako, as co-supervisor, supervised the laboratory work, vetted the results and undertook critical revision of the manuscript.
- Akebe L. K. Abia, as co-supervisor, supervised the laboratory work, vetted the results and undertook critical revision of the manuscript.
- Linda A. Bester aided in the laboratory work and undertook critical revision of the manuscript.
- Sabiha Y. Essack, as the principal supervisor, co-conceptualized the study, guided the literature review and ethical approval application, facilitated data collection and analysis, and undertook critical revision of the manuscript.

Objective(s) met: This paper addresses objectives 1, 2 and 3.

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MULTIDRUG-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI FROM THE UMGUNGUNDLOVU DISTRICT OF KWAZULU-NATAL PROVINCE IN SOUTH AFRICA: EMERGING PATHOGENS.

Jonathan Asante[*1,2], Bakoena A. Hetsa[1,2], Daniel G. Amoako[1], Akebe L. K Abia[1], Linda A. Bester[3], Sabiha Y Essack[1]

[1] Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

[2] School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, South Africa.

[3] Biomedical Research Unit, University of KwaZulu-Natal, South Africa.

*correspondence to: Jonathan Asante

B. Pharm, M. Pharm

Antimicrobial Research Unit

College of Health Sciences

University of KwaZulu-Natal

Private Bag X54001, Durban, 4000

South Africa

Telephone: +27734553105

Email: josante33@yahoo.com

ORCID: https://orcid.org/0000-0001-9637-7328

ABSTRACT

Coagulase-negative staphylococci (CoNS) are increasingly associated with nosocomial infections, especially among the immunocompromised and those with invasive medical devices, posing a significant concern. We report on clinical multidrug-resistant CoNS from the uMgungundlovu District of KwaZulu-Natal Province in South Africa as emerging pathogens.

One hundred and thirty presumptive CoNS from blood culture CoNS isolates constituted the sample. Culture, biochemical tests and the Staphaurex™ Latex Agglutination Test were used for the initial identification of CoNS isolates, while confirmation and speciation were undertaken by the VITEK 2 system. Susceptibilities of isolates against a panel of 20 antibiotics were determined using the Kirby-Bauer disk-diffusion method and the multiple antibiotic resistance (MAR) indices of the isolates were determined. The polymerase chain reaction (PCR) was used to amplify the *mecA* gene to confirm methicillin resistance.

Overall, 89 out of the 130 presumptive CoNS isolates were confirmed as CoNS by the VITEK 2 system. Of this number, 68 (76.4%) isolates were putatively methicillin-resistant by the phenotypic cefoxitin screen test, of which 63 (92.6%) were confirmed by detection of the *mecA* gene. *Staphylococcus epidermidis* (19.1%), *S. hominis* ssp *hominis* (15.7%), and *S. haemolyticus* (16.9%) were the most common CoNS species. Isolates showed high levels of resistance against penicillin (100.0%), erythromycin (74.2%) and azithromycin (74.2%), while displaying high susceptibilities to linezolid (95.5%), gentamicin (95.5%), and tigecycline (94.4%). Multidrug resistance was observed in 76.4% of isolates. MAR index calculation revealed 71.9% with MAR index > 0.2 and 20.2% > 0.5. Isolates with the highest MAR indices (0.7 and 0.8) were recovered from the neonatal intensive care unit. Fifty-one MDR antibiograms were observed.

The high prevalence of methicillin resistance and multidrug resistance in several species of CoNS necessitates surveillance of this emerging pathogen, currently considered a contaminant of microbial cultures.

Keywords: Coagulase-negative staphylococci, antibiotic resistance, multidrug resistance, infections, multiple antibiotic resistance index.

Running title: Coagulase-negative staphylococci in South Africa.

1.0 INTRODUCTION

Staphylococci are classified as either coagulase-positive or coagulase-negative, depending on their ability to clot plasma that is facilitated by the enzyme coagulase (Gómez-Sanz et al., 2019). Coagulase-negative staphylococci (CoNS) are the most frequent colonisers of the skin and mucous membranes and the most frequently isolated organisms in microbiology laboratories (Asante et al., 2020). Although CoNS are mostly considered contaminants in clinical specimens, they have been implicated in clinically relevant infections, including urinary tract infections, endocarditis, bloodstream infections (including neonatal sepsis) and foreign body-related infections (Asante et al., 2020). The skin and mucous membranes of the host, which are home to an abundance of CoNS species, are significant sources of endogenous CoNS infections, facilitated by transmission through medical procedures (Becker et al., 2014).

Pathogenic CoNS are usually associated with clinical environments and found in immunocompromised patients as well as in patients with indwelling metallic or polymer devices, such as orthopaedic prostheses, peripheral venous catheters and artificial pacemakers but are less commonly involved in community-associated diseases (David and Elliott, 2015, Becker et al., 2014, García et al., 2004, Iweriebor et al., 2013). CoNS are the most common pathogens implicated in nosocomial bloodstream infections, responsible for 30-40% of these infections (David and Elliott, 2015). A review found a CoNS prevalence of 6-68% in suspected human infections in Africa within the last decade (Asante et al., 2020). Of the CoNS, *S. epidermidis* is the most common cause of human infection, culpable for about 24-80% of these infections (Becker et al., 2014).

CoNS are noted for their ability to develop antibiotic resistance against commonly used antibiotic classes such as β-lactams, aminoglycosides and macrolides, with particularly high reported methicillin resistance rates (Asante et al., 2020) as well as resistance to antibiotics of last resort such as the glycopeptides (May et al., 2014). Antibiotic resistance genes conferring resistance to these antibiotic classes can be transferred between staphylococcal species such as *S. aureus* and *S. intermedius*, limiting the therapeutic options available (Gómez-Sanz et al., 2019).

This study describes the incidence of MDR CoNS from three hospitals in the uMgungundlovu District in the KwaZulu-Natal Province in South Africa.

2.0 MATERIALS AND METHODS

2.1 ETHICAL CONSIDERATIONS

The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference: BREC/00001302/2020). This study was a sub-study of the overarching research programme on Antibiotic Resistance and One Health (Reference: BCA444/16).

2.2 STUDY SETTING, SAMPLE COLLECTION AND IDENTIFICATION

A total of one hundred and thirty (130) suspected staphylococcal isolates recovered from routine clinical specimens processed by the central microbiology laboratory for uMgungundlovu district over the period October 2019 and February 2020 constituted the sample. Isolates were obtained from blood cultures from both outpatients and inpatients, the latter from the intensive care unit (ICU), neonatal intensive care unit (NICU), paediatric ward, paediatric outpatient department (OPD), emergency departments, surgical ward and nursery.

Presumptive identification was undertaken by Gram staining, colony morphology on blood agar and the catalase test. The Staphaurex™ Latex Agglutination Test (Thermo Scientific, Kent, UK) was used to differentiate staphylococci based on their coagulase activity. Speciation was undertaken using the automated VITEK 2 system (BioMérieux, Marcy-L'Etoile, France). Demographic data of patients (age, sex, ward type, and specimen source) were obtained from anonymous patient records. Isolates were stored at -86°C in tryptic soy broth (Basingstoke, Hampshire, England) containing 10% glycerol (VWR Lifescience Biotechnology, USA) and used for further analyses.

2.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING AND DETERMINATION OF METHICILLIN-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI (MRCONS)

Antibacterial susceptibility profile of isolates against a selected antibiotic panel was ascertained by the Kirby-Bauer disk-diffusion method and interpreted according to the Clinical and Laboratory Standard Institute (CLSI) (Clinical and Laboratory Standards Institute, 2016) guidelines using the following antibiotic discs; penicillin G (10 µg), cefoxitin (30 µg), ceftaroline (30 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), azithromycin (15 µg), erythromycin (15 µg), gentamicin (120 µg), amikacin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), doxycycline (30 µg), teicoplanin (30 µg), tigecycline (15 µg), linezolid (30 µg), clindamycin (10 µg), rifampicin (5 µg), sulphamethoxazole/trimethoprim (1.25/23.75 µg) and nitrofurantoin (300 µg). The cefoxitin test (disc diffusion) was used to screen methicillin resistance (Clinical and Laboratory Standards Institute, 2016). All discs were purchased from Oxoid (Oxoid Ltd, Hampshire, UK). Multidrug resistance was defined as resistance to at least one agent in three or more distinct antibiotic drug classes. Susceptibility testing for vancomycin was done by minimum inhibitory concentration (MIC) (according to the CLSI guidelines) using the broth microdilution method due to the absence of breakpoints for the disc diffusion method (Clinical and Laboratory Standards Institute, 2016). *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 were used as the control strains. The multiple antibiotic resistance (MAR) index was calculated using the formula; MAR=x/y, where x is the number of antibiotics an isolate displayed resistance towards and y is the total number of antibiotics tested against the isolate. The MAR index was used as an indicator of health risk assessment to identify if isolates originate from environments of high or low antibiotic use.

2.4 DNA EXTRACTION AND PCR AMPLIFICATION OF *MecA*

Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) according to the manufacturer's instructions. The purity and concentration of extracted DNA were determined by Nanodrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and stored at -20°C for PCR. PCR detection of the *mecA* gene was done for presumptive methicillin-resistant coagulase-negative staphylococci (MRCoNS) isolates. The *mecA* gene, conferring resistance to methicillin, was amplified using the T100™ Thermal cycler (Bio-Rad, California, USA), using the primer set F-AACAGGTGAATTATTAGCACTTGTAAG and R-

ATTGCTGTTAATATTTTTTGAGTTGAA (Inqaba Biotech, Pretoria, South Africa) (Martineau et al., 2000), generating a 174 base pair fragment (Asante et al., 2019).

PCR was performed in a 25 µl reaction mixture containing 12.5 µl DreamTaq Green PCR Master Mix (ThermoScientific, California, USA), 0.5 µl each of forward and reverse primers, and 3 µl of template DNA. The PCR protocol was denaturation at 94°C for 5 min; 35 cycles of 94°C for 30s; 55°C for 45 s and 72°C for 45 s; and a final extension step of 10 min at 72°C. PCR products were run on 1.5% agarose gel at 120V for 60 min in a 1X Tris-acetate-EDTA (TAE) buffer (BioConcept Ltd, Basel, Switzerland) and visualised by UV transillumination using Bio-Rad ChemiDoc™ MP System (Bio-Rad, California, USA). *S. aureus* ATCC 43300 was used as the positive control.

2.5 STATISTICAL ANALYSIS

Statistical analysis was carried out using Microsoft Excel 2016 and IBM SPSS 26. Possible relationships between variables were investigated using the Pearson Chi-Square test and one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered statistically significant.

3.0 RESULTS

3.1 ISOLATION, IDENTIFICATION AND SPECIES DISTRIBUTION

Eighty-nine (89) out of the 130 presumptive isolates were confirmed as CoNS by the automated VITEK 2 system and used for further analysis. Of these, *Staphylococcus epidermidis*, 17 (19.1%) was the most frequent species identified. Identities of other CoNS isolates were: *S. hominis* ssp *hominis*, 14 (15.7%), *S. haemolyticus*, 15 (16.9%), and *S. lentus*, 13 (14.6%) (Fig 1). Of the remaining 41 non-CoNS isolates, *Enterococcus faecalis* (12), *Enterococcus faecium* (8), *S. aureus* (6) and *Aerococcus viridans* (6) were the most abundant. Others were

Leuconostoc mesenteroides ssp *cremoris* (5), *Enterococcus columbae* (1) and *Dermacoccus nishinomiyaensis* (1).

3.2 ANTIBIOTIC RESISTANCE PHENOTYPIC PATTERNS OF CONS AND *MecA* DETECTION

Of the 89 isolates, 68 (76.4%) were putatively methicillin-resistant by the phenotypic cefoxitin screen test. All isolates displayed resistance to at least one agent in one antibiotic class. High levels of antibiotic resistance rates were recorded for penicillin (100.0%), erythromycin (74.2%), azithromycin (74.2%), cefoxitin (76.4%), and trimethoprim/sulphamethoxazole (68.5%). Isolates were highly susceptible to linezolid (95.5%), gentamicin (95.5%), tigecycline (94.4%), nitrofurantoin (92.1%), amikacin (89.9%), vancomycin (86.5%), teicoplanin (82.0%), and ceftaroline (76.4%). Generally, MRCoNS isolates displayed higher resistance against most antibiotics. Furthermore, MRCoNS showed somewhat reduced susceptibilities of 95.6%, 94.1%, 94.1%, 92.6%, 89.7%, 86.8% and 73.5% against linezolid, tigecycline, gentamicin, nitrofurantoin, amikacin, teicoplanin and ceftaroline, respectively. The detailed phenotypic and genotypic profile of isolates is available in the supplementary Table S1. Table 1 delineates the percentage resistance of all CoNS and MRCoNS isolates to the different antibiotics tested. None of the isolates were resistant to all antibiotics tested. Sixty-three (92.6%) of the 68 MRCoNS by cefoxitin phenotypic test were confirmed as MRCoNS by PCR detection of the *mecA* gene.

3.3 MULTIDRUG RESISTANCE AND MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX

The MAR index ranged from 0.05 to 0.80, with an overall mean of 0.34 (Tables 2 and 3). Multidrug resistance was observed in 68 (76.4%) of the isolates. Fifty-one antibiograms were observed (Table 4).

3.4 DEMOGRAPHIC CHARACTERISTICS OF PATIENTS AND STATISTICAL ANALYSIS

Patients' ages ranged from 0 to 77 years, with the mean age being 17.19 ± 24.33 years. More than half (50.6%) of isolates were obtained from patients who were less than one year old. Isolates were obtained from 46 (51.7%) males and 33 (37.1%) females, while 7 (7.9%) were unknown/unspecified. Outpatients and inpatients made up 19.1% and 80.9% of samples, respectively. The distribution of isolates by wards was as follows; paediatric ward (17.9%), Nursery (6.7%), emergency unit (5.7%), ICU (13.5%), medical ward (11.2%), surgical ward (6.7%), extension ward (7.9%), obstetrics and gynaecology ward (1.1%), and maternity ward (1.1%). The Pearson Chi-Square test showed no significant association between the ward type and MAR index: X^2(104, N=89) =116.05, P=0.197), even though the isolates with the highest MAR indices were from the ICU (Fig 2). Also, there was no statistically significant association between CoNS species and MAR index; X^2(182, N=89) =203.07, P=0.136). Furthermore, even though one-way ANOVA showed that *S. saprophyticus* isolates had a higher MAR mean (p=0.012), the effect size was small (0.201).

4.0 DISCUSSION

This study describes the species distribution, antibiotic resistance profiles, MDR and the MAR indices of clinical CoNS isolates from hospitals in the uMgungundlovu District of KwaZulu-Natal Province in South Africa. There was a diversity of CoNS species isolated, with *S. epidermidis* as the most abundant species, in agreement with previous studies conducted in ICUs that investigated vancomycin heteroresistance and reduced glycopeptide susceptibility among bloodstream CoNS in Egypt and Italy (Mashaly and El-Mahdy, 2017, Natoli et al., 2009). The MRCoNS prevalence rate (76.4%) is higher than that found in previous studies from clinical samples in healthcare settings in Nigeria (46.3%) (Ibadin et al., 2017) and Egypt (75.9%) (Mashaly and El-Mahdy, 2017). However, higher MRCoNS prevalence figures of 86% (Ballot et al., 2012) and 100% (Ehlers et al., 2018) were detected for CoNS implicated in infections in South Africa. The *S. epidermidis* group (*S. epidermidis* and *S. haemolyticus*) are important causes of nosocomial infections (Gómez-Sanz et al., 2019). *S. epidermidis* is the most frequently isolated staphylococcal species in humans and considered the most important CoNS species (Becker et al., 2014). In this study, the *S. epidermidis* group was part of the three most abundant CoNS species.

There was a 92.6% agreement between phenotypic and genotypic confirmation of methicillin resistance in this study. Methicillin resistance in isolates that lack the *mecA* gene may be mediated by other mechanisms of methicillin resistance, such as possession of *mecC* (Dhaouadi et al., 2019), *mecB* genes (Becker et al., 2018), or the overproduction of β-lactamases (Asante et al., 2020). The development of methicillin resistance has been observed in about 80% of CoNS species, contributing to increased morbidity and mortality in hospitals due to their prominence in healthcare-associated infections (HAIs) (Ibadin et al., 2017).

The majority (76.4%) of the isolates in this study showed a multidrug resistance phenotype, with isolates displaying high resistance to commonly used antibiotics such as penicillin (100.0%), macrolides (74.2% each for erythromycin and azithromycin), and sulphamethoxazole/trimethoprim (68.5%). Similar high resistance patterns have been observed against these antibiotics in other studies elsewhere (Adeyemi et al., 2010, Klingenberg et al., 2007). They, however, displayed high susceptibility against reserve antibiotics such as linezolid (95.5%) and the anti-MRSA β-lactam antibiotic ceftaroline (76.4%). Similarly, complete susceptibility of CoNS isolates was observed against vancomycin, tigecycline, teicoplanin and linezolid in another South Africa study (Ehlers et al., 2018). The high susceptibilities recorded against these antibiotics could be due to the reserved use of those antibiotics, mainly for resistant staphylococcal infections. Thus, the last resort antibiotics still retain high activity against CoNS and can be used for empirical treatment of conditions such as suspected CoNS sepsis, even though resistance against these antibiotics is gradually increasing (Asante et al., 2020). Due to its ability to penetrate biofilm, rifampicin is one of the preferred antibiotics for treating bone and joint infections (Nicolosi et al., 2020). However, the development of resistance due to continued use of the antibiotic has necessitated its use in combination with other antibiotics in the treatment of bone and joint infections (Nicolosi et al., 2020). The resistance to rifampicin observed in this study (42.7%) means that the drug may not be relied upon alone in treating infections caused by CoNS.

Taking into consideration their susceptibility profiles, it can be stated that vancomycin, nitrofurantoin, linezolid, tigecycline, teicoplanin, gentamicin, amikacin, and the anti-MRSA cephalosporin ceftaroline retain high activities against CoNS in the study setting and may be relied upon in the treatment of CoNS infections. Decreasing vancomycin susceptibility is reported with increasing frequency in the clinically relevant CoNS literature and may be associated with increased vancomycin exposure (Center et al., 2003). The current study recorded 13.5% CoNS isolates with intermediate susceptibility to vancomycin. It is imperative to mitigate the development of vancomycin non-susceptibility, considering the vital role of glycopeptides in the treatment of resistant infections.

Multidrug resistance (MDR) in CoNS is problematic in low/middle-income countries due to the limited access to newer antibiotics and the high cost of alternative treatment (Asante et al., 2020). Not surprisingly, MRCoNS isolates displayed higher antibiotic resistance compared to methicillin-susceptible CoNS (MSCoNS), as methicillin resistance has been shown to co-select for resistance to other antibiotics (Asante et al., 2020, Pyörälä and Taponen, 2009). The study showed that the majority of isolates were multidrug resistant, with 71.9% of isolates having MAR indices of >0.20 and 18 (20.2%) had MAR values of ≥ 0.50. Other studies have as well recorded high MDR rates of CoNS (Ibadin et al., 2017). MAR values higher than 0.2 are indicative of isolates, possibly originating from environments where antibiotics are frequently used and may also hint at possible nosocomial transmission within the hospital setting (Paul et al., 1997).

There was no statistically significant association between the type of ward and the MAR index (P=0.197), even though some isolates with high MAR indices were recovered in the neonatal ICU.

Considering that CoNS are recognised neonatal pathogens in upper and high-income countries, they ought to be given equal attention in low- and middle-income countries (Patel and Saiman, 2010). That the ICU had the highest number of recovered isolates is significant as CoNS are frequently isolated in bloodstream infections in ICU patients (Asante et al., 2020). The use of invasive devices such as catheters, commonly used in ICUs, increases the risk of infection by CoNS, that is further facilitated by biofilm formation.

CONCLUSION

The study reports relatively high levels of methicillin and multidrug resistance among CoNS isolates with a wide range of MAR indices. Considering that CoNS naturally inhabit the skin and mucous membranes, they may only be contaminants of clinical specimens. However, they are increasingly emerging pathogens, necessitating due diligence when recovered from clinical specimens.

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Table 1 Antimicrobial susceptibility pattern of CoNS and MRCoNS isolates from clinical sources

Antibiotic	**CoNS isolates (n=89)**			**MRCoNS isolates(n=68)**		
	NO OF SUSCEPTIBLE (%)	NO OF INTERMEDIATE (%)	NUMBER OF RESISTANT (%)	NO OF SUSCEPTIBLE (%)	NO OF INTERMEDIATE (%)	NUMBER OF RESISTANT (%)
Cefoxitin	21 (23.6)	NA	68 (76.4)	0 (0.0)	NA	68 (100.0)
Penicillin G	0 (0)	NA	89 (100)	0 (0.0)	NA	68 (100.0)
Ceftaroline	68 (76.4)	12 (13.5)	9 (10.1)	50 (73.5)	12 (17.6)	6 (8.8)
Ciprofloxacin	41 (46.1)	10 (11.2)	38 (42.7)	25 (36.8)	7 (10.3)	36 (52.9)
Moxifloxacin	46 (51.7)	6 (6.7)	37 (41.6)	30 (44.1)	5 (7.4)	33 (48.5)
Azithromycin	19 (21.3)	4 (4.5)	66 (74.2)	8 (11.8)	3 (4.4)	57 (83.8)
Erythromycin	17 (19.1)	6 (6.7)	66 (74.2)	6 (8.8)	5 (7.4)	57 (83.8)
Gentamicin	85 (95.5)	2 (2.2)	2 (2.2)	64 (94.1)	2 (2.9)	2 (2.9)
Amikacin	80 (89.9)	5 (5.6)	4 (4.5)	61 (89.7)	3 (4.4)	4 (5.9)
Chloramphenicol	64 (71.9)	2 (2.2)	23 (25.8)	47 (69.1)	2 (2.9)	19 (27.9)
Tetracycline	62 (69.7)	4 (4.5)	23 (25.8)	44 (64.7)	3 (4.4)	21 (30.9)
Doxycycline	65 (73.0)	3 (3.4)	21 (23.6)	46 (67.4)	2 (2.9)	20 (29.4)
Teicoplanin	73 (82.0)	10 (11.2)	6 (6.7)	59 (86.8)	4 (5.9)	5 (7.4)
Tigecycline	84 (94.4)	NA	5 (5.6)	64 (94.1)	NA	4 (5.9)
Linezolid	85 (95.5)	NA	4 (4.5)	65 (95.6)	NA	3 (4.4)
Clindamycin	47 (52.8)	10 (11.2)	32 (35.9)	32 (47.1)	7 (10.3)	29 (42.6)
Rifampicin	51 (57.3)	0 (0.0)	38 (42.7)	34 (50.0)	0 (0.0)	34 (50.0)
Sulphamethoxazole/trimethoprim	24 (26.9)	4 (4.5)	61 (68.5)	16 (23.5)	3 (4.4)	49 (72.1)
Nitrofurantoin	82 (92.1)	2 (2.2)	5 (5.6)	63 (92.6)	2 (2.9)	3 (4.4)
Vancomycin*	77 (86.5)	13 (13.5)	0 (0.0)	62 (91.2)	6 (8.8)	0 (0.0)

*Susceptibility against vancomycin was determined using the broth microdilution method.

Table 2 Multiple antibiotic resistance (MAR) index of CoNS isolates

MAR INDEX	Number of isolates
0.05	5 (5.6%)
0.10	5 (5.6%)
0.15	10 (11.2%)
0.20	5 (5.6%)
0.25	10 (11.2%)
0.30	6 (6.7%)
0.35	8 (8.9%)
0.40	12 (13.5%)
0.45	10 (11.2%)
0.50	8 (8.9%)
0.55	2 (2.2%)
0.60	6 (6.7%)
0.70	1 (1.1%)
0.80	1 (1.1%)

Table 3 Distribution of CoNS isolates depending upon MARI value >0.2 in various departments

Department	**Number of isolates with MAR index >0.2 (n=64)**	**Percentage**
Emergency	5	7.8%
ICU	11	17.2%
Medical Ward	10	15.6%
Obstetrics/gynaecology	1	1.6%
OPD	7	10.9%
Surgical Ward	5	7.8%
Extension Ward	7	10.9%
Paediatric Ward	9	14.1%

Table 4 Resistance pattern observed in MDR CoNS (n=68)

Resistance pattern	**Number**
FOX-PEN-CIP-MXF-AZM-ERY-GEN-CLI-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-CLI-RIF-SXT	3
PEN- AZM-ERY-CHL-CLI-SXT	1
PEN-CPT-CHL-RIF-NIT	1
FOX-PEN-CIP-MXF-AZM-ERY-CLI-RIF	4
FOX-PEN-CIP-MXF-AZM-ERY-GEN-DOX-RIF-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-AMK-CLI-RIF	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-TET-DOX-TGC-TEC-LZD-CLI-RIF-SXT-NIT	1
FOX-PEN-MXF-AZM-ERY-CHL-CLI	1
PEN-AZM-ERY-SXT	3
FOX-PEN-AZM-ERY-SXT	6
PEN-CPT-MXF- CHL-TET-DOX-TGC-TEC-LZD-RIF-SXT-NIT	1
FOX-PEN-CIP-MXF-AZM-ERY-CLI-RIF-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-TET-DOX-RIF-SXT	2
FOX-PEN-AMK-CHL-TET-DOX-TGE-TGC-LZD-RIF-SXT-NIT	1
FOX-PEN-CPT-CIP-MXF-AZM-ERY-CHL-TET-DOX-CLI-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-TET-DOX-TGC-TEC-LZD-RIF-SXT-NIT	1
PEN- CIP-MXF-AZM-ERY-CHL-CLI-RIF-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-TET-DOX-CLI-RIF-SXT	2
FOX-PEN-MXF-AZM-ERY-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-AMK-CHL-CLI-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-TET-CLI-RIF	1
FOX-PEN-AZM-ERY-CLI-RIF-SXT	1
FOX-PEN-CPT-MXF-AZM-ERY-TET-DOX-CLI-RIF-SXT	1
FOX-PEN-CPT-CIP-MXF-AZM-ERY-AMK-CHL-CLI-RIF-SXT	1
FOX-PEN-CIP-MXF-AZM-RIF-SXT	1
FOX-PEN-AZM-ERY-CHL-TET-DOX-SXT	2
PEN-MXF-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-CLI-RIF-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-TET-DOX-CLI-RIF-SXT	1

FOX-PEN-AZM-ERY-TET-CLI	1
PEN-CIP-MXF-AZM-ERY-TET-CLI-RIF-SXT	1
FOX-PEN-CIP-AZM-ERY-TET-DOX-RIF-SXT	1
FOX-PEN-TET-DOX-TGC-RIF	1
FOX-PEN-AZM-ERY-RIF	1
FOX-PEN-CPT-AZM-ERY-TET-DOX-SXT	2
FOX-PEN-AZM-ERY-CHL-SXT	1
FOX-PEN-AZM-ERY-CHL-CLI-RIF-SXT	2
FOX-PEN-CIP-MXF-AZM-ERY-CLI-SXT	1
FOX-PEN-CIP-AZM-ERY-TET-DOX-SXT	1
FOX-PEN-CPT-CIP-AZM-ERY-CLI-RIF-SXT	1
FOX-PEN-CIP-AZM-ERY-TET-DOX	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-TET-DOX-SXT	1
FOX-PEN-CIP-AZM-ERY-CLI-SXT	1
FOX-PEN-CIP-MXF-ERY-RIF	1
FOX-PEN-AZM-ERY-TEC-RIF-SXT	1
FOX-PEN-AZM-ERY-TEC	1
FOX-PEN-CIP-MXF-AZM-ERY-CHL-CLI-SXT	1
FOX-PEN-CIP-MXF-AZM-ERY-RIF	1
FOX-PEN-CIP-MXF-RIF	1

Abbreviations: FOX, cefoxitin; PEN, penicillin G; CPT, ceftaroline; CIP, ciprofloxacin; MXF, moxifloxacin; AZM, azithromycin; ERY, erythromycin; GEN, gentamicin; AMK, amikacin; CHL, chloramphenicol; TET, tetracycline; DOX, doxycycline; TEC, teicoplanin; TGC, tigecycline; LZD, linezolid; CLI, clindamycin; RIF, rifampicin; SXT, sulphamethoxazole/trimethoprim; NIT, nitrofurantoin

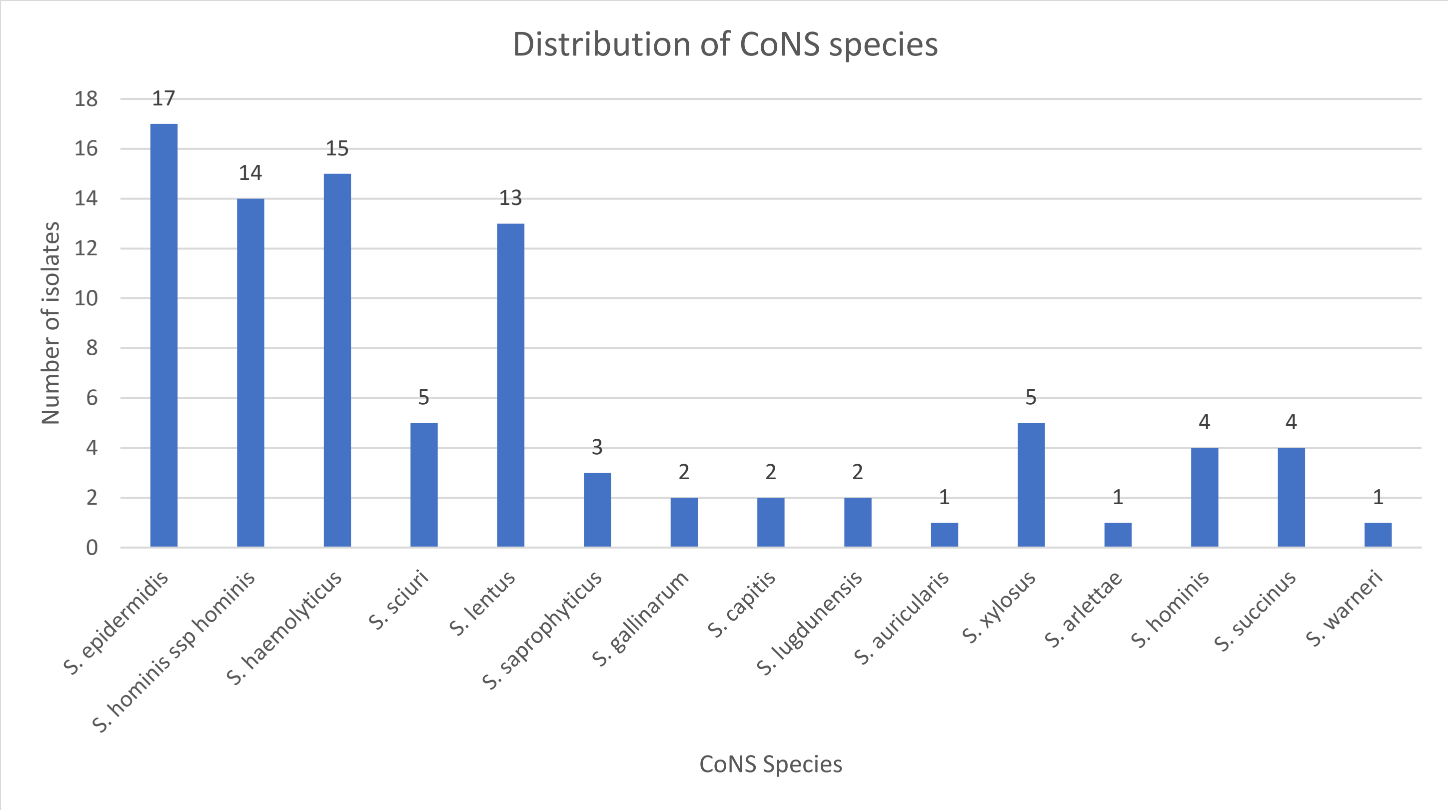

Figure 1 Distribution of CoNS isolates in this study

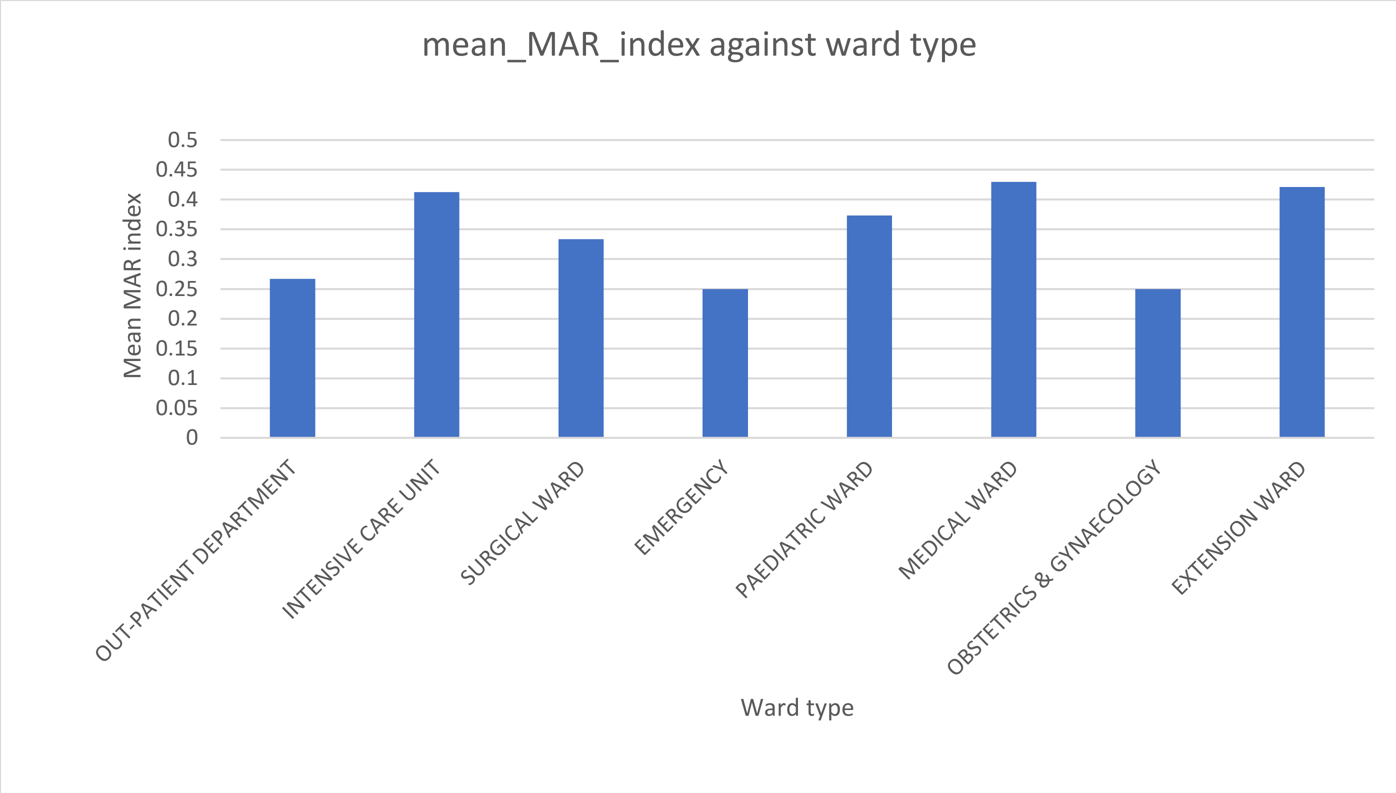

Figure 2 Bar chart showing the distribution of MAR index against various ward types

Table S1 Detailed phenotypic and *mecA* profile of isolates

Isolate ID	**CoNS species**	**Ward type**	*MecA*	**Antibiotic resistance profile**																		
				FOX	PEN	CPT	CIP	MXF	AZM	ERY	GEN	AMK	CHL	TET	DOX	TEC	TGC	LZD	CLI	RIF	SXT	NIT
C1	*S. epidermidis*	Paediatric OPD	NA		R																R	
C3	*S. epidermidis*	Ward & nursery	-	R	R		R	R	R	R	R								R		R	
C4	*S. epidermidis*	A1 paediatric ward	-	R	R		R	R	R	R			R						R	R	R	
C5	*S. hominis ssp hominis*	Paediatric OPD	NA		R				R	R			R						R		R	
C6	*S. epidermidis*	Emergency department	NA		R	R							R							R		R
C7	*S. haemolyticus*	3N ICU	+	R	R		R	R	R	R									R	R		
C9	*S. epidermidis*	-	+	R	R		R	R	R	R	R				R					R	R	
C10	*S. haemolyticus*	ICU	+	R	R		R	R	R	R		R							R	R		
C11	*S. hominis ssp hominis*	Casualty	NA		R																R	
C13	*S. hominis ssp hominis*	Neonatal ICU	+	R	R		R	R	R	R			R	R	R	R	R	R	R	R	R	R
C17	*S. hominis ssp hominis*	H1 Medical ward	+	R	R			R	R	R			R						R			
C18	*S. haemolyticus*	KMMC Clinic	NA		R				R	R											R	
C19	*S. epidermidis*	Neonatal ICU	+	R	R				R	R											R	
C20	*S. hominis ssp hominis*	H2 Medical ward	NA		R	R		R					R	R	R	R	R	R		R	R	R
C21	*S. sciuri*	ICU	+	R	R		R	R	R	R									R	R	R	
C22	*S. lentus*	Ward D	+	R	R		R	R	R	R				R	R					R	R	
C23	*S. lentus*	Paediatric OPD	NA		R																	
C26	*S. lentus*	-	+	R	R																	
C27	*S. haemolyticus*	Ward D	+	R	R							R	R	R	R	R	R	R		R	R	R
C31	*S. haemolyticus*	A1 Paediatric ward	+	R	R				R	R												
C32	*S. sciuri*	E2 Paediatric ICU	+	R	R		R	R	R	R									R	R		
C35	*S. saprophyticus*	E1 Paediatric ward	+	R	R	R	R	R	R	R			R	R	R				R		R	
C36	*S. saprophyticus*	Neonatal ICU	+	R	R		R		R	R			R	R	R	R	R	R		R	R	R
C37	*S. lentus*	Neonatal ICU	NA		R		R	R	R	R			R						R	R	R	

C38	*S. epidermidis*	H2 Medical ward	+	R	R		R	R	R	R											R	
C39	*S. lentus*	5B2 Medical ward	+	R	R		R	R	R	R			R	R	R				R	R	R	
C40	*S. hominis ssp hominis*	3N Extension ward	+	R	R			R	R	R											R	
C42	*S. haemolyticus*	5F Medical ward	+	R	R		R	R	R	R				R	R					R	R	
C43	*S. lentus*	D1 Medical ward	+	R	R		R	R	R	R		R	R						R		R	
C44	*S. gallinarum*	WARD E	+	R	R																	
C47	*S. hominis ssp hominis*	D1 Medical ward	+	R	R				R	R											R	
C48	*S. hominis ssp hominis*	3F Obstetrics & Gynaecology ward	+	R	R				R	R											R	
C49	*S. capitis*	3N Extension ward	+	R	R		R	R	R	R				R					R	R		
C53	*S. lentus*	3N Main	-	R	R				R	R									R	R	R	
C54	*S. haemolyticus*	3N Extension ward	+	R	R		R	R	R	R									R	R		
C55	*S. lentus*	Ward & Nursery	NA		R				R	R												
C56	*S. lentus*	E1 Paediatric ward	+	R	R	R		R	R	R				R	R				R	R	R	
C57	*S. epidermidis*	7B2 Extension ward	-	R	R	R	R	R	R	R		R	R						R	R	R	
C58	*S. epidermidis*	Surgical OPD	+	R	R		R	R	R	R			R	R	R				R	R	R	
C61	*S. haemolyticus*	D1 Medical ward	+	R	R		R	R	R											R	R	
C66	*S. lentus*	Paediatric OPD	NA		R	R															R	
C68	*S. epidermidis*	7F Paediatric ward	+	R	R				R	R			R	R	R						R	
C72	*S. lentus*	2R Surgical ICU	NA		R																	
C73	*S. lentus*	Ward F	NA		R																R	
C74	*S. epidermidis*	Paediatric OPD	NA		R			R													R	
C75	*S. lugdunensis*	-	NA		R																	
C81	*S. hominis ssp hominis*	F2 Surgical ward	+	R	R		R	R	R	R									R	R	R	
C87	*S. auricularis*	3N Main	+	R	R				R	R				R					R			

C93	*S. lentus*	H1 Medical ward	+	R	R		R	R	R	R			R						R	R	R	
C100	*S. haemolyticus*	Emergency department	+	R	R				R	R											R	
C102	*S. haemolyticus*	Paediatric OPD	+	R	R		R	R	R	R				R	R				R	R	R	
C104	*S. capitis*	7F Paediatric ward	NA		R		R	R	R	R				R					R	R	R	
C105	*S. hominis ssp hominis*	Paediatric OPD	NA	R	R		R		R	R				R	R					R	R	
C107	*S. lugdunensis*	-	NA		R																	
C110	*S. xylosus*	H2 Medical ward	+	R	R									R	R		R			R		
C113	*S. xylosus*	Medical OPD	+	R	R				R	R										R		
C114	*S. sciuri*	Medical OPD	NA		R				R	R												
C116	*S. hominis ssp hominis*	Paediatric OPD	NA		R				R	R											R	
C118	*S. hominis ssp hominis*	Ward D	+	R	R	R			R	R				R	R						R	
C119	*S. xylosus*	2F Paediatric ICU	+	R	R	R			R	R				R	R						R	
C120	*S. gallinarum*	Paediatric OPD	+	R	R				R	R			R								R	
C121	*S. hominis ssp hominis*	E1 Paediatric ward	+	R	R																R	
C122	*S. hominis ssp hominis*	Paediatric OPD	+	R	R																R	
C123	*S. haemolyticus*	Emergengy And Accident Unit	NA		R				R	R												
C125	*S. arlettae*	KMMC Clinic	+	R	R				R	R			R						R	R	R	
C126	*S. epidermidis*	1F Male surgical Ward	NA		R																	
C127	*S. hominis*	1F Male Surgical ward	+	R	R				R	R											R	
C128	*S. hominis*	1F Male surgical Ward	+	R	R		R	R	R	R									R		R	
C129	*S. haemolyticus*	1F Male Surgical ward	+	R	R		R	R	R	R									R	R		
C131	*S. sciuri*	Emergency and Accident Unit	+	R	R		R		R	R				R	R						R	
C132	*S. sciuri*	C2 Surgical ward	+	R	R	R	R		R	R									R	R	R	
C133	*S. epidermidis*	Paediatric OPD	+	R	R																R	
C134	*S. epidermidis*	Casualty	+	R	R		R		R	R				R	R							

C135	*S. saprophyticus*	Paediatric OPD	+	R	R		R	R	R	R			R	R	R						R	
C136	*S. succinus*	H Ward	NA		R				R	R											R	
C137	*S. epidermidis*	Ward O	+	R	R		R		R	R									R		R	
C138	*S. hominis*	H Ward	+	R	R																R	
C139	*S. succinus*	3N Extension ward	+	R	R				R	R			R						R	R	R	
C141	*S. haemolyticus*	3N Extension ward	+	R	R		R	R		R										R		
C142	*S. succinus*	Paediatric OPD	+	R	R																R	
C143	*S. warneri*	3N ICU	+	R	R				R	R						R				R	R	
C144	*S. epidermidis*	3N Extension ward	+	R	R		R	R	R	R			R						R	R	R	
C145	*S. epidermidis*	Casualty	+	R	R				R	R						R						
C146	*S. haemolyticus*	Paediatric ward	+	R	R															R	R	
C147	*S. xylosus*	Ward and Nursery	+	R	R		R	R	R	R			R						R		R	
C148	*S. xylosus*	D5 Ward	+	R	R				R	R												
C149	*S. hominis*	KMMC Clinic	+	R	R				R	R											R	
C150	*S. succinus*	Paediatric OPD	+	R	R				R	R			R	R	R						R	
C151	*S. haemolyticus*	E1 Paediatric Ward	+	R	R		R	R	R	R										R		
C152	*S. haemolyticus*	ICU	+	R	R		R	R												R		

Abbreviations: FOX, cefoxitin; PEN, penicillin G; CPT, ceftaroline; CIP, ciprofloxacin; MXF, moxifloxacin; AZM, azithromycin; ERY, erythromycin; GEN, gentamicin; AMK, amikacin;

CHL, chloramphenicol; TET, tetracycline; DOX, doxycycline; TEC, teicoplanin; TGC, tigecycline; LZD, linezolid; CLI, clindamycin; RIF, rifampicin; SXT, sulphamethoxazole/trimethoprim;

NIT, nitrofurantoin; R, resistant; OPD, Outpatient Department; ICU, Intensive Care Unit; +, present; -, absent; NA: Not applicable

CHAPTER 4 - MANUSCRIPT 3

GENOMIC ANALYSIS OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATES FROM CLINICAL SOURCES IN THE KWAZULU-NATAL PROVINCE, SOUTH AFRICA.

AUTHOR CONTRIBUTIONS

- **Jonathan Asante**, as the principal investigator, co-conceptualized the study, undertook the laboratory work and drafted the manuscript.
- Bakoena A. Hetsa, aided with analysis and undertook critical revision of the manuscript.
- Daniel G. Amoako, as co-supervisor, supervised the laboratory work, vetted the results and undertook critical revision of the manuscript.
- Akebe L. K. Abia, as co-supervisor, supervised the laboratory work, vetted the results and undertook critical revision of the manuscript.
- Linda A. Bester aided in the laboratory work and undertook critical revision of the manuscript.
- Sabiha Y. Essack, as the principal supervisor, co-conceptualized the study, guided the literature review and ethical approval application, facilitated data collection and analysis, and undertook critical revision of the manuscript.

Objective(s) met: This paper addresses objectives **4**, **6** and **7**.

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GENOMIC ANALYSIS OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATES FROM CLINICAL SOURCES IN THE KWAZULU-NATAL PROVINCE, SOUTH AFRICA.

Jonathan Asante[*1,2], Bakoena A. Hetsa[1,2], Daniel G. Amoako[1], Akebe L. K Abia[1], Linda A. Bester[3], Sabiha Y Essack[1]

[1] Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

[2] School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, South Africa.

[3] Biomedical Research Unit, University of KwaZulu-Natal, South Africa.

*correspondence to: Jonathan Asante

B. Pharm, M. Pharm

Antimicrobial Research Unit

College of Health Sciences

University of KwaZulu-Natal

Private Bag X54001, Durban, 4000

South Africa

Telephone: +27734553105

Email: josante33@yahoo.com

ORCID: https://orcid.org/0000-0001-9637-7328

ABSTRACT

Background: *Staphylococcus epidermidis* has become an important nosocomial pathogen. Multidrug resistance makes *S. epidermidis* infections difficult to treat. Although considered less virulent than its coagulase-positive counterpart, *S. epidermidis* is an essential carrier of mobile genetic elements and antibiotic resistance genes that can be transferred within and between staphylococcal species.

Methods: Sixteen methicillin-resistant *S. epidermidis* (MRSE) underwent whole-genome sequencing and bioinformatics analyses were carried out to ascertain their resistome, virulome, mobilome, clonality and phylogenomic relationships.

Results: In all, 75% of isolates displayed multidrug resistance and were associated with the carriage of multiple resistance genes including *mecA*, *blaZ*, *tet(K)*, *erm(A)*, *erm(B)*, *erm(C)*, *dfrG*, *aac(6')-aph(2'')* and *cat(pC221)* conferring resistance to β-lactams, tetracyclines, macrolide-lincosamide-streptogramin B, aminoglycosides and phenicols, that were located on both plasmids and chromosomes. Their virulence profiles were evidenced by the presence of genes involved in adherence/biofilm formation (*icaA*, *icaB*, *icaC*, *atl*, *ebh*, *ebp*), immune evasion (*adsA*, *capC*, *manA*) and antiphagocytosis (*rmlC*, *cdsA*, *cpsA*). The community-acquired SCC*mec* type IV was the most common SCC*mec* type. The CoNS belonged to seven multilocus sequence types (MLSTs) and carried a diversity of mobile genetic elements such as phages, insertion sequences and plasmids. The bacterial anti-phage defense systems CRISPR-Cas immunity phage system and Restriction-Modification System (R-M system) and the Arginine Catabolic Mobile Element (ACME) involved in immune evasion and transport of virulence genes were also found. The insertion sequence, IS256, linked with virulence, was found in 56.3% of isolates. Isolates were organised into two major clades with some similarity but also considerable variability within isolates.

Conclusion: Whole-genome sequencing and bioinformatics analysis provide insights into the likely pathogenicity and antibiotic resistance of *S. epidermidis*, necessitating surveillance of this emerging pathogen.

Keywords: *Staphylococcus epidermidis*, antibiotic resistance, whole-genome sequencing, genomics.

Running title: Genomics of *Staphylococcus epidermidis* in South Africa

1.0 INTRODUCTION

Staphylococcus epidermidis are coagulase negative staphylococci (CoNS) that are commensals of the skin microbiome. Among the CoNS, *S. epidermidis* and *S. haemolyticus*, together referred to as the *S. epidermidis group*, are the most prevalent in clinical settings (Azih and Enabulele, 2013) and can behave as pathogens by colonising medical devices, infecting surgical wounds, and causing bacteraemia (Cabrera-Contreras et al., 2019). CoNS infection is commonly associated with device-associated healthcare infection. *S. epidermidis* is considered clinically relevant, moderately pathogenic and is known to display multidrug and methicillin resistance that complicates treatment (Becker et al., 2014, Xu et al., 2015). The *mecA* gene, which mediates methicillin resistance, is highly conserved in *S. epidermidis*, easily transferred to other staphylococcal species by horizontal transmission (Méric et al., 2015) and borne on the mobile genetic element (MGE) staphylococcal cassette chromosome, SCC*mec*, which is shared between *S. epidermidis* and *S. aureus* (Méric et al., 2015). It is estimated that globally, *S. epidermidis* together with other coagulase-negative staphylococci (CoNS) and *S. aureus*, cause 30% of hospital-associated infections (Xu et al., 2018).

S. epidermidis is an important carrier of antibiotic resistance genes (ARGs), which can be transferred between staphylococcal species (Xu et al., 2018). The pathogenicity of *S.*

epidermidis is further enhanced by virulence genes associated with adherence/biofilm formation, phenol-soluble modulins and various MGEs such as plasmids, insertion sequences (ISs), transposons, pathogenicity islands and phages that are involved in the acquisition and transmission of resistance and virulence characteristics (Bouchami et al., 2016, Rolo et al., 2017). The arginine catabolic mobile element (ACME) system, a pathogenicity island thought to facilitate host colonisation and immune evasion has generated interest in recent years (O'Connor et al., 2018). According to recent phylogenetic studies, the ACME most likely originated from *S. epidermidis* and transmitted to *S. aureus* through horizontal transfer (Onishi et al., 2013, Planet et al., 2013).

Other factors that have been associated with pathogenicity in *S. epidermidis* include the metabolic state of the bacterial cell, genomic rearrangements in pathogenic isolates facilitated by IS256 and the conjugative transfer of antibiotic resistance (Cabrera-Contreras et al., 2019).

The widespread sequence types ST5, ST12 and ST23 have been reported to be exhibit high resistance against most antibiotic drug classes (Martínez-Meléndez et al., 2016). Additionally, there is increasing rifampicin resistance in *S. epidermidis* isolates belonging to ST2 and ST23 in Europe, the USA and Australia (Lee et al., 2018). This observed resistance conferred by mutations in the *rpoB* gene have independently emerged, supporting the assertion that few, well-adapted clonal lineages of *S. epidermidis* abound in clinical environments (Lee et al., 2018).

In this study, we describe the genomic features of methicillin-resistant *S. epidermidis* (MRSE) isolated from hospitals within the KwaZulu-Natal province in South Africa, specifically their resistome, virulome, mobilome, clonality and phylogenies together with associations between them and other parameters.

2.0 MATERIALS AND METHODS

2.1 ETHICAL APPROVAL

Ethical approval for the study was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal under reference number BREC/00001302/2020. This study was a sub-study of the overarching research programme on Antibiotic Resistance and One Health (Reference: BCA444/16).

2.2 DESCRIPTION OF STRAINS AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

Sixteen MRSE isolates were selected as a sub-sample from the initially identified and screened 89 CoNS isolates and subjected to whole-genome sequencing. *S. epidermidis* isolates were mainly selected because of their clinical relevance, known for their role as the most frequent cause of nosocomial infections and their well-documented ability to form biofilms. The sixteen clinical MRSE isolates collected from patients in three hospitals in the uMgungundlovu District in the KwaZulu-Natal Province, South Africa, had been subjected to initial identification by Gram staining, colony characteristics and the Staphaurex™ Latex Agglutination Test (Thermo Scientific, Kent, U.K). Speciation of isolates was done using the automated VITEK 2 system (BioMérieux, Marcy-L'Etoile, France).

The antibiotypes for 20 antibiotics were determined by the Kirby-Bauer disk-diffusion method, or by the broth microdilution method. The Clinical and Laboratory Standard Institute (CLSI) guidelines were used to interpret their antimicrobial susceptibility (Clinical and Laboratory Standards Institute, 2016). Antibiotics tested (disc diffusion) included penicillin G (10 µg), cefoxitin (30 µg), ceftaroline (30 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), azithromycin (15 µg), erythromycin (15 µg), gentamicin (120 µg), amikacin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), doxycycline (30 µg), tigecycline (15 µg), teicoplanin (30 µg), linezolid (30 µg), clindamycin (10 µg), rifampicin (5 µg), sulphamethoxazole/trimethoprim (1.25/23.75 µg) and nitrofurantoin (300 µg). The minimum inhibitory concentration (MIC) of vancomycin was determined by the broth microdilution method according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2016). The cefoxitin disc diffusion was used to detect methicillin resistance and molecularly confirmed by PCR detection of the *mecA* gene.

2.3 PHENOTYPIC DETECTION OF BIOFILM FORMATION

Biofilm forming abilities of isolates were assessed by the tissue culture plate method described by Mitchell et al. (2010) (Mitchell et al., 2010). Briefly, isolates were grown in trypticase soy broth (TSB) supplemented with glucose for 24h at 37°C. Sterile 96-well microtiter plates were inoculated with bacterial suspension adjusted to 0.5 MacFarland standard and incubated at 37°C for 24h. The plates were washed after incubation and dried at room temperature. The wells then stained with 0.1% crystal violet solution, incubated at room temperature for 10 minutes and washed thrice with distilled water. A 30% acetic acid solution was added to solubilize the crystal violet retained by the biofilm. The optical densities of samples in each well were read at 570 nm using a microtiter plate reader (BMG LABTECH, Offenburg, Germany). *Staphylococcus epidermidis* ATCC 35984 was used as positive control. Isolates were categorised as strong, moderate, weak and non-biofilm formers.

2.4 WHOLE-GENOME SEQUENCING

Genomic DNA (gDNA) from pure colonies of MRSE isolates grown from overnight cultures was extracted and purified using the GenElute Bacterial Genomic DNA kit (Sigma Aldrich, St. Louis, USA), according to the manufacturer's instructions. DNA was checked by agarose gel electrophoresis, while the concentration and purity were determined using Nanodrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). For library preparation, the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA, USA) was used to generate paired-end libraries, followed by whole-genome sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at the Sequencing Core Facility, National Institute for Communicable Disease, Johannesburg, South Africa.

Quality trimming of the sequence reads was done by the use of Sickle version 1.33 (https://github.com/najoshi/sickle), while assembler, SPAdes version 3.11 (Bankevich et al., 2012) and the CLC Genomics Workbench version 10.1 (CLC, Bio-QIAGEN, Aarhus, Denmark) were used for de novo assembly of the reads. The assembled contiguous sequences were submitted via the NCBI Prokaryotic Genome Annotation Pipeline to GenBank for gene annotation. The generated contigs were analysed further to investigate genetic elements of interest.

2.5 BIOINFORMATIC ANALYSES

2.5.1 PATHOGENICITY, RESISTOME AND VIRULOME ANALYSIS

The prediction of isolates' pathogenicity towards human hosts was determined by PathogenFinder available at https://cge.cbs.dtu.dk/services/PathogenFinder/. The assembled genomes from the WGS data were annotated to predict and identify the resistome using ResFinder 4.1 (with a minimum length and threshold of 60% and 90%, respectively), and the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/analyze/rgi) (Alcock et al., 2020), using the default selection criteria 'perfect and strict hits only'. The platforms were used side by side to compensate for the inherent deficits in individual platforms.

We determined the genetic basis (chromosomal single nucleotide polymorphism [SNP]) for observed fluoroquinolone and rifampicin resistance from the assembled genomes by investigating mutations conferring resistance to fluoroquinolones and rifampicin using BLASTn. Briefly, the *gyrA*, *gyrB*, *parC*, *parE* and *rpoB* genes in a reference susceptible *S.*

epidermidis (*Staphylococcus epidermidis* strain ATCC 12228) were aligned with the corresponding genes from resistant isolates in this study with BLASTn to call for SNPs in those genes using the Clustal Omega tool (European Molecular Biology Laboratory). Thus, the mutations in the genomes of the study isolates were manually curated.

VirulenceFinder 2.0 (using a minimum length of 60% and a threshold of 90%) (https://cge.cbs.dtu.dk/services/VirulenceFinder/) (Joensen et al., 2014), virulence factor database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFanalyzer) and BacWGSTdb (http://bacdb.cn/BacWGSTdb) were used to screen for the presence of virulence genes. Various virulence determinants consistent with different major virulence factors (including adherence, enzymes, immune evasion, secretion system, toxins, anti-phagocytosis, biofilm formation/adherence and intracellular survival) associated with *S. epidermidis* were investigated.

2.5.2 IN SILICO MULTILOCUS SEQUENCE TYPING (MLST)

MLST was performed in silico using MLST 2.0 program software (https://cge.cbs.dtu.dk/services/MLST/) available on the website of the Center for Genomic Epidemiology (Larsen et al., 2012) and the pubic molecular typing database, PubMLST (https://pubmlst.org/). Sequence types were assigned by matching the internal fragments of the seven housekeeping genes (*arcC, aroE, gtr, mutS, pyrR, tpiA* and *yqiL*) from *S. epidermidis* to identify alleles (Thomas et al., 2007). We performed eBURST analyses (Feil et al., 2004) in the MLST database to identify clones similar to obtained STs.

2.5.3 IDENTIFICATION OF MOBILE GENETIC ELEMENTS (MGES) AND GENETIC SUPPORT ENVIRONMENT

MGEs associated with antibiotic resistance genes and their genetic context were investigated using NCBI annotations. The rapid annotation using Subsystem Technology (RAST 2.0) (Aziz et al., 2008) was used to ascertain MGEs and the genetic support environment. The web-based typing tool SCCmecFinder (https://cge.cbs.dtu.dk/services/SCCmecFinder/) was used for the in silico determination of the SCC*mec* types and their structural position in the MRSE isolates. In silico detection of plasmid replicon types was done out using PlasmidFinder 2.1, available at https://cge.cbs.dtu.dk/services/PlasmidFinder/ (Carattoli et al., 2014).

The **PHA**ge **S**earch **T**ool **E**nhanced **R**elease (PHASTER) (available at https://phaster.ca/) was used to identify and annotate prophage sequences within the genomes (Arndt et al., 2016). Only the prophage regions identified as 'intact' by PHASTER were considered. The region positions of the prophages were BLASTED on CARD to determine if the prophages harboured resistance genes. Insertion sequences (ISs) and transposons flanking the resistance genes were identified using the MobileElementFinder v1.0.3 (2020-10-09) (https://cge.cbs.dtu.dk/services/MobileElementFinder/) (Johansson et al., 2020), available on the website of the Center for genomic epidemiology (https://cge.cbs.dtu.dk/services/). We used NCBI annotations to determine the support environment of the resistance genes.

2.5.4 CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS/CRISPR-ASSOCIATED (CRISPR/CAS SYSTEM), ARGININE CATABOLIC MOBILE ELEMENT (ACME) AND RESTRICTION-MODIFICATION SYSTEM (RMS)

We searched for CRISPR and cas genes in the sequence data using the CRISPRCasFinder, available at https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index, using the default advanced settings for CRISPR and the clustering model 'SubTyping' for cas. Restriction-ModificationFinder 1.1, available at https://cge.cbs.dtu.dk/services/Restriction-ModificationFinder/, was used to investigate the presence of the R-M system, using a minimum length of 60% and a threshold for %ID of 95% (Roer et al., 2016). The ACME genes within the genomes were detected and aligned. Alignment of the ACME components made up of the *arc* operon, the *opp3* operon, and the *kdp* operon, was used to classify the ACME components as follows: *arc* and *opp3* operons (type I), the *arc* operon only (type II), the *opp3* operon only (type III), the *arc* and *kdp* operons (type IV), and all three *arc*, *opp* and *kdp* operons (type V), using Pathosystems Resource Integration Center (PATRIC) (https://www.patricbrc.org/) annotations.

2.5.6 PHYLOGENETIC ANALYSES USING WGS SNPs AND WGS MLST TREES

Phylogenetic trees were constructed based on the maximum likelihood method using the CSIPhylogeny (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) (Kaas et al., 2014) which performs SNP calling, filtering of the SNPs, and inferring phylogeny based on the concatenated alignment of the high-quality SNPs, using the assembled contigs. The analysis was performed on the platform using default parameters as follows: minimum depth at SNP positions, 10X; minimum relative depth at SNP positions of 10%; minimum distance between SNPs (prune) at 10bp; minimum SNP quality, 30; minimum read mapping quality of 25 and minimum Z-score of 1.96. *Staphylococcus aureus* ATCC 25923 was used as the reference strain. Assembled genomes for comparison were uploaded. To see how our isolates compare to *S. epidermidis* genomes from Africa, we searched and downloaded *S. epidermidis* genomes reported in Africa and curated on the PATRIC website and included them in the analysis. To edit and visualize the phylogenetic tree, we used the Figtree programme (http://tree.bio.ed.ac.uk/software/figtree/). We used Phandango (Hadfield et al., 2018) to visualize the phylogeny in association with the isolate demographics, resistance mechanisms, in silico WGS typing metadata.

2.6 NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The nucleotide sequences of the 16 MRSE strains (C35, C36, C38, C40, C68, C81, C119, C122, C127, C133, C135, C137, C138, C145, C146 and C148) used in this work were uploaded in GenBank database in the Bioproject number PRJNA667485, with the following accession numbers: **JADPYQ000000000, JADPYL000000000, JADPYK000000000, JADPYJ000000000, JADPYN000000000, JADPYP000000000, JADBPJ000000000, JADPYR000000000, JADPYO000000000, JADBPI000000000, JADPYI000000000, JADWON000000000, JADBPA000000000, JADBPG000000000, JADWOP000000000, JADBPQ000000000.**

3.0 RESULTS

3.1 PHENOTYPIC CHARACTERISTICS OF MRSE ISOLATES

Sixteen blood culture MRSE isolates were used in the WGS analysis. All isolates were confirmed as methicillin-resistant by PCR detection of the *mecA* gene. Twelve (75%) were multidrug-resistant, defined as resistance to at least one antibiotic in three or more distinct antibiotic classes (Magiorakos et al., 2012). Two isolates were resistant to the anti-MRSA cephalosporin ceftaroline, while one and two isolates were resistant to tigecycline and teicoplanin, respectively. None of the isolates were resistant to vancomycin; however, one isolate showed intermediate susceptibility to vancomycin, while one isolate each was resistant to linezolid and nitrofurantoin. Antibiotic susceptibility profiles of MRSE isolates used in this study are shown in Table 1.

3.2 GENOME AND ASSEMBLY CHARACTERISTICS

The genome and assembly characteristics of the sequences, including size, number of contigs, number of RNAs, GC content (%), number of coding sequences, N_{50} and L_{50} are shown in supplementary Table S1. The isolates' draft genome size ranged from 1.9 Mb to 2.9 Mb, with a GC content of 31.7% to 32.5%.

3.3 GENOMIC CHARACTERISATION OF RESISTOME

The identities of isolates and observed phenotypic resistance were confirmed by the genomic data using the Pathogenwatch platform. Antibiotic resistance genes conferring resistance to β-lactams (*mecA*, *blaZ*), tetracyclines [*tet(K), tet(M)*], macrolide-lincosamide-streptogramin B antibiotic (MLS_B) [*erm(A), erm(B), erm(C), msr(A)*, and *mph(C)*], trimethoprim-sulfamethoxazole (*dfrG*), aminoglycosides [*aac(6')-aph(2''), aph(3')-III*, *aadD*], chloramphenicol [*cat(pC221), cat(pC233)*], fosfomycin [*fosB*], were detected in isolates (Table 2). All MRSE isolates possessed either the *mecA* or *blaZ* genes. Resistance determinants for tigecycline, teicoplanin, linezolid and nitrofurantoin were not detected. The mechanism(s) behind these resistance phenotypes are currently under investigation (He et al., 2019, Wardenburg et al., 2019, Yushchuk et al., 2020). There was discordance between resistance phenotype and ARGs, relating to trimethoprim-sulfamethoxazole, tetracycline, doxycycline, rifampicin and erythromycin resistance phenotypes. Even though some isolates were phenotypically resistant to these antibiotics, no corresponding ARGs were detected.

Thirteen out of the 16 isolates showed an agreement between the cefoxitin resistance phenotype and the *mecA* gene. The tetracycline resistance genes *tet(K)* and *tet(M)* were found in 4/5 (80%) of isolates phenotypically resistant to tetracycline. The aminoglycoside resistance mechanisms *aac(6')-aph(2''), aad* and *aph(3')-III* were found in 6/16 (37.5%) of isolates; however none of those isolates were phenotypically resistant to gentamicin or amikacin. Furthermore, the MLS_B resistance mechanisms *msr(A), mph(C), erm(A), erm(B)* and *erm(C)* were also detected in 11/12 (91.6%) of phenotypically resistant isolates. The *dfrG* gene was found in 9/14 (62.3%) of isolates resistant to sulfamethoxazole/trimethoprim. We identified known and putatively novel mutations in the *gryA*, *parC* and *parE* quinolone resistance-determining region (QRDR) genes in some fluoroquinolone-resistant isolates. (Table 3). We detected no mutation in the drug target in one of the three isolates found to be resistant to rifampicin. Resistance, in this case, may be mediated by a mechanism yet to be described. We identified the major facilitator superfamily (MFS) antibiotic efflux pump (*norA*), which can also confer resistance to fluoroquinolones.

Seven mutations were found in *gyrA*, 4 mutations in *parC* but no mutations were detected in *gyrB*. We further found 2 mutations (S486Y and Y737S) in the *rpoB* gene.

3.4 PATHOGENICITY AND VIRULOME

The mean probability of isolates being pathogenic to humans ranged from 0.727-0.968 and matched several pathogenic families. The virulome analysis revealed putative virulence genes encoding proteins belonging to multiple virulence categories of *S. epidermidis*, i.e., adherence/biofilm formation, enzymes, immune evasion, secretion, toxin, anti-phagocytosis, intracellular survival and stress adaptation (Table 4).

3.5 WGS-BASED MULTILOCUS SEQUENCE TYPING (MLST)

In silico MLST analyses identified seven different MLST types, namely sequence types (ST) ST54 (2), ST83 (1), ST2 (2), ST490 (1), ST640 (1), ST210 (1) and ST59 (2). The most resistant isolate belonged to ST54 and harboured nine ARGs encoding resistance to five antibiotic drug classes (Table 2). The observed STs were from three different hospitals with sequence type ST59 (n=2) from the same hospital but different wards. The eBURST analyses matched the various STs to the closest global ancestry STs. ST54 matched STs originating from human and animal sources from Denmark, Italy, Japan, India and Russia. The eBURST analyses also matched ST2 to the highest number of similar clones originating from several countries including Argentina, Cape Verde, Denmark, Germany, Spain, Italy and Japan (Table S2).

3.6 MOBILOME AND THE GENETIC SUPPORT ENVIRONMENT

In silico SCC*mec* typing/subtyping revealed six SCC*mec* types/subtypes: SCC*mec* type IV(2B), SCC*mec* type IVg(2B), SCC*mec* type V(5C2), SCC*mec* type IVa(2B), SCC*mec* type XIII(9A) and SCC*mec* type I(1B). Isolates with SCC*mec* type I(1B) (n=2) belonged to sequence type (ST59), while SCC*mec* type V(5C2) isolates (n=2) belonged to sequence type ST54. The community-acquired SCC*mec* type IV (in various subtypes) was the most common type found. The *mecA* gene (but not *mecC*) was detected by SCCmecFinder as the sole mechanism of resistance in MRSE isolates. All β-lactam resistant isolates possessed the *blaZ* gene (encoding β-lactamase) and their regulator genes *blaR* and *blaI*.

Plasmid analysis by PlasmidFinder and BacWGSTdb (http://bacdb.org/BacWGSTdb/) revealed 24 different plasmid replicon types. Rep10 (10), rep7a (7) and repUS43 (6) were the most predominant plasmid replicon types. Replicon types rep39, repUS9 and rep19b were found in 4 (25%), 4 (25%) and 5 (31.2%) of isolates, respectively.

We found ISs in all 16 isolates. In total, 10 different IS types belonging to 6 different IS families were detected (Fig S1). The most predominant IS families were IS256, 1S200/IS605 and IS3. IS256, closely linked to biofilm formation and virulence in pathogenic MRSE isolates, was found in 9 isolates, 7 of which had the *ica* operon. The resistance gene *aac(6')-aph(2'')* was found in association with IS256, while the virulence gene *gelE* (predicted to be linked with Enterococcus) was found in association with ISEfa11 in one isolate (C81). Furthermore, the resistance gene *erm(A)* was also found in association with the transposon Tn554 in some isolates. Using NCBI annotation, we found the *blaZ* gene surrounded by regulator genes *blaR* and *blaI*. Similarly, the *mecA* gene was frequently found with the regulatory gene *mecI* (a repressor) and *mecR1* (a sensor inducer) and ISs (IS257 and IS1182). Table 5 and Figure 1 describe the genetic support environment of some resistance genes found in this study, with focus on the association MGEs with the ARGs and virulence genes.

The PHASTER tool identified intact prophages integrated into the genomes of 10 isolates. PHAGE_Staphy_StB20 (n=9), and PHAGE_Staphy_187 (n=5) were the most predominant prophages. Prophages did not harbour resistance genes. Prophage characteristics, including GC content and number of coding sequences, are shown in Supplementary Table S3.

3.7 IDENTIFICATION AND CLASSIFICATION OF CRISPR-CAS ELEMENTS, ARGININE CATABOLIC MOBILE ELEMENT (ACME) AND RESTRICTION-MODIFICATION SYSTEM (R-M SYSTEM)

The CRISPRCasFinder identified sequences with clustered regularly interspaced short palindromic repeats (CRISPR). All isolates possessed at least one sequence with CRISPR. However, CRISPR-associated (cas) genes were not detected. Two isolates possessed the R-M system, and both were classified as type II. ACME was identified in 5 isolates and were classified as type I (3) and type III (2).

3.8 PHYLOGENETIC RELATIONSHIP AMONG *S. EPIDERMIDIS* ISOLATES IN THIS STUDY AND WITH OTHER AFRICAN *S. EPIDERMIDIS* ISOLATES

The phylogenetic relationship between the 16 study isolates and 5 collected isolates from African countries, together with a reference strain, was determined (Fig 2a and 2b). The tree consists of two main clades, A and B, defined by the branches. Clade B was further organised into sub-clades B1 and B2, further organized into B2a and B2b, and subsequently into B2bi and B2bii. Generally, isolates did not cluster according to STs, except C36 and C40 (both ST54).

4.0 DISCUSSION

In this study, we sequenced MRSE isolates from clinical sources from hospitals in the uMgungundlovu District in the KwaZulu-Natal Province in South Africa. Using WGS, we studied the genomic characteristics, including resistance and virulence determinants, MGEs and the genetic environments of the resistance genes observed.

Concerning pathogenicity, there is no apparent genetic difference between commensal non-pathogenic and pathogenic *S. epidermidis* strains, albeit nosocomial *S. epidermidis* strains are boosted with resistance and virulence genes (Méric et al., 2015). The 16 genomes herein analysed were mostly from paediatric/neonatal patients. *S. epidermidis* is commonly recovered from bacterial bloodstream infections from neonatal units as a probable causative agent (Asante et al., 2020). Children are particularly susceptible to acquiring *S. epidermidis* in perinatal hospitals (Cabrera-Contreras et al., 2019).

We detected various resistance genes encoding resistance to several antibiotic drug classes that explained the observed phenotypic resistance in isolates. Resistance genes found in this study encode enzyme inactivation (β-lactamases), enzyme modification of antibiotic target such as *erm* genes that mediate macrolide-lincosamide-streptogramin B (MLS_B) resistance, the aminoglycoside-modifying enzymes, replacement of antibiotic target site as in *mecA*-mediated resistance to β-lactams in staphylococci, and major facilitator superfamily (MFS) antibiotic efflux pump (*norA*), which can also confer resistance to fluoroquinolones (Foster, 2017). There was no association between ARG type and hospital/department. However, *mecA*, *blaZ* and *norA* genes were found in nearly all isolates from all three hospitals. As well, *erm(C)*, *msr*(A), *dfrG* and *mph(C)* genes were distributed across the regional hospital but were not ward specific, while *dfrG*, *tet(K)*, and *erm(B)* were frequently found in the district hospital.

Generally, there was considerable agreement between resistance phenotypes and genotypes observed with ARGs affirming the phenotype, except in a few instances. The detection of the β-lactamase gene, *blaZ* and the regulator genes *blaR* and *blaI* are most likely responsible for the resistance to penicillin. The discordance between cefoxitin resistance phenotype and the *mecA* genotype could be due to alternative mechanisms of resistance, hetero-resistance or drawback of the present phenotypic testing methods (Band et al., 2019, Harrison et al., 2019).

The lack of phenotypic resistance to gentamicin and amikacin even though aminoglycoside resistance genes were detected could be due the lack of expression of these genes. This could also be due to the fact that amikacin is not affected by most aminoglycoside-modifying enzymes (Doyle et al., 2020). Other factors that can cause discordance between phenotype and genotype include sequence quality of the sample and the read depth of the sequencing platform; insufficient read depth leads to lower coverage (Doyle et al., 2020). The molecular mechanism of resistance in the tigecycline-resistant isolate in this study is unknown and there was no *tet(X)* gene detected. Similarly, even though resistance phenotypes were observed for teicoplanin, linezolid and nitrofurantoin, molecular resistance mechanisms were not detected in their genomes. Limitations of current phenotypic detection methods could be responsible for such discrepancies (Cabrera-Contreras et al., 2019). Novel resistance mechanisms may explain these observed phenotypes and is subject to further studies (Osei Sekyere and Asante, 2018).

We found mutations in the *gyrA, gyrB, parC, parE* and the *rpoB* genes. In the *gyrA* gene, the substitution S84Y, a mutation known to confer fluoroquinolone resistance (Yamada et al., 2008), was detected, while putatively novel mutations V304I, E888D, D890E, S891D, D892S, E893D were also detected. The effects of the individual mutations on fluoroquinolone resistance were not investigated in this study.

Several virulence genes are shared by both pathogenic and commensal *S. epidermidis* strains (Otto, 2009). Consistent with hospital and commensal *S. epidermidis* strains isolated worldwide (Cabrera-Contreras et al., 2019), our isolates were characterised by adherence/biofilm forming genes and multidrug resistance. Twelve out of the 16 isolates were biofilm formers as determined by the phenotypic tissue culture plate method, most of which were corroborated by the detection of genes involved in adherence. The *ica* operon and IS256 used as measures of pathogenicity in *S. epidermidis* (Murugesan et al., 2018) were not detected in 9 and 7 isolates, respectively. The isolates, however, cannot be dismissed as non-pathogenic, as they can deploy several other virulence factors such as immune evasion (encoded by *hasC, rfbA-1, adsA, capC*), toxins (encoded by *hlb, hlyA, cysC1*) anti-phagocytosis (*rmlC, cdsA, cpsA, cpsF*), stress adaptation (*katA, katA, mntB, sodCI*) and intracellular survival (*lplA1*).

S. epidermidis form biofilms on medical devices and on biotic surfaces that can lead to the breakaway of single cells, spreading and colonising other parts of the body, leading to infections such as endocarditis and sepsis. Biofilm formation allows *S. epidermidis* to persist at the infection site and beyond and form a physical barrier to antibiotics. Thus, strains with biofilm-forming ability are considered more virulent (Becker et al., 2014). The *ica* operon, which facilitates the adherence of staphylococci to components of the host extracellular matrix, plays a part in the formation of biofilm (Ghasemian et al., 2015). Furthermore, *S. epidermidis* possesses other determinants known to facilitate attachment to surfaces and promote various biofilm formation stages. These genes include the elastin binding protein gene *ebp*, serine protease genes *sspA*, autolysin gene *atlE*, lipase genes *geh*, the cell wall-associated fibronectin-binding protein gene *ebh* and the nuclease gene, *nuc* (Xu et al., 2018).

These antibiotic resistance and virulence genes have been shown to form part of the accessory genome organised within and between species. Prediction of isolates' pathogenicity towards human hosts yielded a high average probability score ($P_{score} \approx 0.937$). This pathogenicity score juxtaposed with the several virulence genes possessed by isolates, support their pathogenic potential to humans (Adzitey et al., 2020).

Methicillin-resistant staphylococci have been associated with MGEs, such as the SCC*mec* and ACME. MGEs may be repositories of resistance and virulence genes (Foster, 2017, Sheppard et al., 2016). Their importance is related to their mobile nature, which allows them to transfer from cell to cell, within and between bacterial species through horizontal gene transfer, resulting in frequent exchange of genetic material within the population. The variability observed in the genome of *S. epidermidis* points to the active gene exchange. We thus looked for prophages, CRISPR-Cas system, transposons and insertion sequences to investigate this phenomenon. The study isolates possessed various ISs, that belong to different families. IS256 has been found to be present in pathogenic *S. epidermidis* strains and closely linked with virulence and biofilm formation among MRSE (Murugesan et al., 2018). This observation was affirmed by the fact that all but 2 of our isolates with IS256 also contained the *ica* operon involved in biofilm formation. Indeed, only one isolate (C133) harboured the *ica* gene that did not have IS256. IS256 has been shown to facilitate genomic rearrangements in pathogenic *S. epidermidis* isolates (Cabrera-Contreras et al., 2019).

In this study, the plasmids rep10, repUS43, rep7a, rep7b, repUS70 frequently carried the resistance genes *erm(C)*, *tet(M)*, *tet(K)*, *catpC233*, and *blaZ*, respectively, whereas other genes were chromosomally-mediated. These plasmid-borne genes can easily be transferred by conjugation between cells, spreading resistance (Cabrera-Contreras et al., 2019). Furthermore, although prophages can transfer DNA between cells by transduction, no resistance genes were carried by prophages detected in this study. Transposons detected in the genome of 2 isolates, like plasmids, may carry genes beneficial to bacteria, such as those involved in antibiotic resistance (Mbelle et al., 2019). They can transpose from the chromosome and can move to different sites of the DNA within a cell. In this study, the transposon Tn554 was found to flank the resistance gene *erm(A)* in some isolates, which may allow it to jump between the chromosome and the plasmid. The small sample size of isolates in this study limits associations between ARGs, virulence genes, MGEs and isolate demographics. However, the carriage of ARGs on diverse MGEs enhances the mobilization and dissemination of these genes.

CRISPR-Cas system is a defense mechanism deployed by bacteria against phage infection. After surviving a viral infection, certain bacteria imprint a piece of the viral genetic code as a memory of the infection. Bacteria may use this to neutralise future infections caused by similar viruses by cleaving the viral genetic sequence before they can take control of the bacterial host (Makarova et al., 2020). All isolates bore at least one sequence with CRIPSR (Table 2), ten of which contained at least one intact prophage. The two isolates found with the R-M system were classified as type II. Like the CRISPR-Cas system, the R-M system is a defense system developed by bacteria against invasion by bacteriophages (Vasu and Nagaraja, 2013).

The ACME system, a pathogenicity island, has generated interest and is thought to facilitate the host colonisation and immune evasion and transports virulence or survival genes (O'Connor et al., 2018). ACME elements were detected in 31.3% of study isolates. This is lower than the prevalence of 40 to 65.4% reported in MRSE in a study that investigated the diversity of the ACME in *S. epidermidis* from the oral cavity and periodontal pockets (O'Connor et al., 2018). In comparison, an ACME carriage of 16% was detected in a study that compared the resistance and virulence profile of *S. epidermidis* isolates from bloodstream infections and nares of neonates (Salgueiro et al., 2017). ACME shows a higher prevalence and greater diversity in *S. epidermidis* compared with *S. aureus*. In *S. aureus*, studies have proved that ACME is usually incorporated in bacterial chromosome adjoining the SCC*mec*IV element (Diep et al., 2006, Ellington et al., 2008). In this study however, no association between ACME and SCC*mec* type IV was found, which is consistent with results obtained by Du *et al*. (2013). Most of the resistance genes were bracketed by either transposases or ISs or a combination of both and these can transfer resistance genes within and between plasmids and chromosomes (Mbelle et al., 2019) potentially within and between bacterial species.

The *cat(pC233)* gene was bracketed by plasmid mobilization relaxosome protein MobC in isolate C36, while the *erm(C)* gene was bracketed by the 23S rRNA methylase leader peptide ErmCL and replication/maintenance protein RepL. In two isolates (C148 and C119), the *blaZ* gene was surrounded by the type I toxin-antitoxin system, which may play a role in biofilm and persister cell formation (Wang and Wood, 2011). (Table 5 and Fig 1).

MLST has shown the population structure of *S. epidermidis* to be clonal (Thomas et al., 2007). The clonal lineages ST2, ST5, and ST23, which are the most commonly reported in hospital environments as well as other sequence types of *S. epidermidis* are globally distributed (Miragaia, 2018). ST2 in particular, is predominant in the hospital environment. In this study, 2/16 isolates belonged to ST2. Both ST2 isolates in this study possessed the *icaA* gene and IS256, both of which are linked to enhanced pathogenicity (Du et al., 2013). Also, ST35, ST81 and ST89 were not represented in this study, consistent with global data (Cabrera-Contreras et al., 2019). Despite the relatedness of isolates, there is still considerable variation within individual isolates pointing to their mobilization on diverse MGEs.

5.0 Conclusion

S. epidermidis isolates from public hospitals in uMgungundlovu exhibited several permutations and combinations of ARGs, virulence genes and MGEs pointing to a complex milieu of mobilized antibiotic resistance and pathogenic characteristics in clonal and multiclonal strains.

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Table 1 Antibiotic susceptibility characteristics of the MRSE collected from blood cultures

Isolate ID	**Sex**	**Antibiotic resistance profile**																			
		FOX	PEN	CPT	CIP	MXF	AZM	ERY	GEN	AMK	CHL	TET	DOX	TGC	TEC	LZD	CLI	RIF	SXT	NIT	VAN
C35	F	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	S	R	S	S
C36	M	R	R	S	R	I	R	R	S	I	R	R	R	R	R	R	I	R	R	R	S
C38	F	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S
C40	M	R	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S
C68	M	R	R	S	I	S	R	R	S	S	R	R	R	S	S	S	S	S	R	S	S
C81	F	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	S	S
C119	M	R	R	R	S	S	R	R	S	S	S	R	R	S	I	S	S	S	R	S	I
C122	M	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
C127	M	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S
C133	M	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
C135	M	R	R	S	R	R	R	R	S	S	R	R	R	S	S	S	S	S	R	S	S
C137	U	R	R	S	R	I	R	R	I	S	S	S	S	S	S	S	R	S	R	S	S
C138	F	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
C145	M	R	R	S	S	S	R	R	S	S	S	S	S	S	R	S	S	S	I	S	S
C146	M	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
C148	F	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S

Abbreviations FOX, cefoxitin; PEN, penicillin; CPT, ceftaroline; CIP, ciprofloxacin; MXF, moxifloxacin; AZM, azithromycin; ERY, erythromycin; GEN, gentamicin; AMK, amikacin; CHL, chloramphenicol; TET, tetracycline; DOX, doxycycline; TGC, tigecycline; TEC, teicoplanin; LZD, linezolid; CLI, clindamycin; RIF, rifampicin; SXT, sulphamethoxazole/trimethoprim; NIT, nitrofurantoin; VAN, vancomycin; R; resistant; I, intermediate; S, susceptible; M, male; F, female; U, unknown.

Antibiotic susceptibility tests were interpreted according to the CLSI breakpoints for coagulase-negative staphylococci.

Table 2 Genotypic characteristics of the MRSE isolates

Isolate	**Resistome (Plasmid/Chromosomal-mediated)**	**Plasmid replicon type**	**R-M system**	**SCC*mec* type**	**ACME type**	**MLST**	**Insertion sequences**	**No. of CRISPR-Cas elements**	**Pathogenicity score (no. of pathogenic families)**
C35	*mecA, blaZ, norA, dfrG, tet(K), cat(pC221), erm(C),*	rep15, rep19b, rep19c, rep24c, rep39, rep21, repUS22, rep7a, repUS43, rep10	-	SCC*mec* type IV(2B)	-	unknown	IS256, ISSau4, ISSep3	10(0)	0.944(482)
C36	*MecA, blaZ, tet(M), aac(6')-aph(2''), aadD, cat(pC221),cat(pC233), erm(A),erm(C), norA*	rep19b, repUS9, rep10, rep22, rep7a, rep7b, repUS43	Type II	SCC*mec* type V(5C2)	-	ST54	IS256	5(0)	0.942(540)
C38	*MecA, blaZ, norA, erm(C)*	rep10, repUS43	Type II	SCC*mec* type IV(2B)	-	ST83	ISSep3, ISSau4, IS256	3(0)	0.943(555)
C40	*MecA, blaZ, aac(6')-aph(2''), erm(A),erm(C)*	rep10, repUS9, repUS43	-	-	-	ST54	IS256	4(0)	0.944(521)
C68	*MecA, blaZ, tet(M),tet(K), cat(pC221), erm(C),lsa(A),erm(B), dfrG*	rep2, repUS11, rep10, rep7a, repUS43, repUS12	-	SCC*mec* type XIII(9A)	III	ST210	ISSau4, ISEfa11	6(0)	0.947(501)
C81	*MecA, blaZ, norA, aac(6')-aph(2''), cat(pC221), erm(A),erm(C),erm(B)*	rep7a, repUS43, rep2, repUS11, rep22, repUS46, repUS23, repUS9, rep10	-	SCC*mec* type IV(2B)	-	ST2	ISEfa11, ISSep3, ISSau4, IS256	5(0)	0.942(531)
C119	*mecA, blaZ, erm(C), aac(6')-aph(2''), aac(6')-Ic, dfrG*	rep10, rep19b, rep19c, rep20, rep39, repUS70	-	-	III	unknown	IS16, ISSep2, IS256	6(0)	0.947(171)
C122	*mecA, blaZ, aadD, aac(6')-aph(2''), mph(C), msr(A), dfrG*	rep19b, rep19c, rep20, rep39, rep10,	-	SCC*mec* type I(1B)	I	ST59	ISSep3, ISSau4	3(0)	0.951(513)
C127	*mecA, blaZ, mph(C), msr(A), erm(C), dfrG*	rep10, rep19b, rep19c, rep20, rep39	-	SCC*mec* type I(1B)	I	ST59	ISSau4, ISSep3	4(0)	0.949(548)
C133	*blaZ*	None	-	-	-	ST490	ISSep3	4(0)	0.964(152)
C135	*mecA, blaZ, norA, tet(K), msr(A), mph(C), cat(pC221), dfrG*	rep7a, repUS70	-	SCC*mec* type IVg(2B)	I	unknown	ISSep3	3(0)	0.953(394)

C137	*mecA, norA, blaZ, aac(6')-aph(2''), erm(A),erm(C), dfrG*	rep10, repUS9, repUS43	-	-	-	unknown	IS256	1(0)	0.727(36)
C138	*blaZ, dfrG*	-	-	-	-	unknown	ISSep3, ISSau4	7(0)	0.955(328)
C145	*MecA, blaZ, tet(K), aac(6')-aph(2''), aph(3')-III, erm(B)*	rep11a, rep18b, rep2, rep7a	-	SCC*mec* type IVa(2B)	-	ST2	ISSep3, ISSau4, ISEnfa3, IS16, ISEfa5, IS256	3(0)	0.968(1)
C146	*MecA, blaZ, msr(A)*	repUS48	-	SCC*mec* type IVa(2B)	-	ST640	IS30, ISEc36, ISSep2, ISSau4	4(0)	0.948(121)
C148	*blaZ, tet(K)*	rep21, rep19c, repUS9, rep5d, rep7a	-	-	-	unknown	IS256	2(0)	0.948(30)

RM-System: Restriction-Modification System; ACME-arginine catabolic mobile element: ACME type I (*arc* and *opp3* operons), II (the *arc* operon only), III (the *opp3* operon only), IV (*arc* and *kdp* operons) and V (*arc*, *opp* and *kdp* operons). Pathogenicity score: Prediction of a bacteria's pathogenicity towards human hosts using PathogenFinder. Strain of the closet pathogenic family linkage: *Staphylococcus epidermidis* ATCC 12228

Table 3 Mutations in the *gyrA*, *gyrB*, *parC* and *parE* in the *S. epidermidis* isolates

Isolate ID	***gyrA***	***gyrB***	***parC***	***parE***	***rpoB***
C35	-	-	-		N/A
C135	-	-	*K272R	NONE	N/A
C137	*V304I	-	*K272R	NONE	N/A
C40	-	-	*K272R	NONE	N/A
C38	S84Y, *E888D, *D890E, *S891D, *D892S, *E893D	-	*S80F, *D84Y, *E231K, *K272R	NONE	N/A
C36[μ]	-	-	*K272R	NONE	-
C81[μ]	-	-	*K272R	D434V	S486Y
C146[#]	N/A	N/A			*Y737S

Mutations in the *gyrA, gyrB, parC* and *parE* in the *S. epidermidis* isolates. *Putatively novel mutations; [#] isolates resistant to rifampicin only

[μ] isolates resistant to both fluoroquinolones and rifampicin.

Table 4 Virulence genes identified in MRSE isolates in this study

Isolate ID	Virulence gene							
	Adherence/biofilm	Enzymes	Immune evasion	Secretion	Toxin	Antiphagocytosis	Intracellular survival	Stress adaptation
C35	*atl, ebh, ebp, sdrE, sdrH, prgB/asc10, dltA,ebpC,pavA,flmH,slrA,plr/gapA, fsrA,fsrB,fsrC*	*geh, lip, sspA, nuc, gelE, EF0818, stp,sprE,*	*hasC, rfbA-1, rmlB, rmlD,capC,manA,*	*esaA,esaD,esaG,essA,essB,essC,esxA*	*hlb*	*rmlC,cdsA,cpsA,cpsF,gnd*	*lplA1*	*katA*
C36	*atl,ebh,ebp,icaA,icaB,icaC,icaR,*	*geh,lip,nuc*	*adsA,capC,manA*	-	*hlb, cylR2*	-	-	
C38	*atl, ebh, ebp, icaA, icaB,icaC,icaR,sdrC,sdrG,sdrH,prgB/asc10,dltA,ebpC,pavA,slrA, fsrA,fsrB,fsrC*	*sspB, geh, lip, sspA, nuc, gelE, EF0818, stp,sprE*	*hasC, rfbA-1, rmlB, rmlD,galE,manA*	*essC*	*hlb*	*rmlC,cdsA,cpsA,cpsF,gnd*	*lplA1*	*katA*
C40	*atl, ebh, clfA, ebp, icaA, icaB,icaC,icaR,sdrG,sdrH,prgB/asc10,dltA,ebpC,pavA,slrA,plr/gapA*	*sspB, geh, lip, sspA, nuc, gelE, EF0818, stp, sprE*	*hasC, rfbA-1, rmlB, rmlD,manA,*	*essC*	*hlb*	*rmlC,cdsA,cpsA,cpsF,gnd*	*lplA1*	*katA*
C68	*atl,ebh,ebp,sdrH,flmH*	*sspB,geh,lip,sspA,nuc*	*capB*	-	*hlb*	-	-	
C81	*atl, ebh, ebp, icaA, icaB, icaC, icaR, sdrG, sdrH, asa1, dltA, ebpC, fss3, pavA,slrA,plr/gapA*	*sspB,geh,lip,nuc,gelE,stp,sprE*	*hasC, rfbA-1, rmlB, rmlD,galE,gtaB,manA*	-	*hlb*	*rmlC,cdsA,uppS,gnd*	-	*katA*
C119	*sdrH*	*sspB,geh,sspA,nuc*	-	-	*hlb*		-	
C122	*atl, ebh, ebp, sdrG, sdrH,hcpB,htpB,orfH,flmH,nueA,tapT,fimC,fimD,fimD,pilU,pilQ, adeG,pgaC*	*sspB,geh,lip,sspA,plcN,eno*	*galE,galU,mrsA/glmM,pgi,acpXL,gtaB*	*flgA, flgB, flgC, flgD, flgE, flgF, clpV1, yplA*	*hlb, hlyA, cysC1*	*algU,rmlB,wbjD/wecB,gnd,manB,uge,wzb,wzc*	-	*katG,katA, mntB,sodCI*
C127	*atl,ebh,ebp,sdrG,sdrH, hcpB,flmH,nueA,fimC,fimD,pilU,pilQ, pgaC*	*sspB,geh,lip,sspA,nuc,plcN,eno,*	*galE,galU,capC*	*flgA,flgE,flgF,flgG,flgJ,flgK,fliC,fliI,fliR,yplA*	*hlb*	*algU,rmlB,wbjD/wecB,cpsG_1,uge,wzc*	-	*katG,katA, mntB*
C133	*atl,ebh,ebp,icaA,icaB,icaC,icaR,sdrC,sdrH-*	*sspB,geh,lip,nuc*	-	-	*hlb*	-	-	

C135	-	*sspB,geh,lip,sspA,nuc*	-	-	*hlb*	-	-	
C137	*ebp, icaA, icaB, sdrF, sdrH,hcpB,htpB,orfH,flmH,nueA,tapT,fimA,fimC,fimD,pilU,pilQ, adeG,pgaC*	*sspB,geh,lip,plcN,eno*	*galE,galU,mrsA/glmM,pgi,acpXL,*	*flgA,flgB,flgC,flgD,flgE,flgF*	*hlb, hlyA, cysC1*	*rmlB,wbjD/wecB,cpsG_1,gnd,uge,wzc*	-	*katG*
C138	-	-	-	-	-	-	-	
C145	*atl,ebh,ebp,icaA,icaB,icaC,icaR,sdrG,sdrH,*	*sspB,geh,lip,sspA,nuc*	-	-	*hlb*	-	-	
C146	*atl, ebh, ebp, sdrG, sdrH, csgG,ecpA,fleR,fliQ,hcpB,htpB,orfH,flgC,flgC,plr/gapA,pilW, pgaC*	*sspB,geh,lip,sspA,eno*	*galE,galU,mrsA/glmM,pgi,acpXL,*	*esaA,esaD,esaE,essB,essC,flgB,flgC,flgD,ipaH,clpV*	*hlb, hlyA, cysC1*	*algU,rmlB,wbjD/wecB,gnd,wcaG,wcaI,wzb*	-	*katG,sodCI*
C148	*atl,ebp,sspB,sspC,geh,sspA*	-	-	-	-		-	

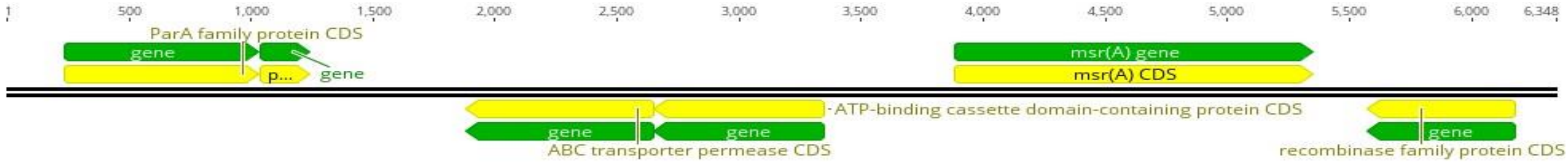
Table 5 MGEs associated with antibiotic resistance genes in the MRSE strains

Isolate	Contig	Synteny	Plasmid/chromosomal sequence with closest nucleotide homology (accession number)
C35	237	*msr(A)*:recombinase family protein	*S. warneri* strain WB224 plasmid pWB224_2 (CP053472.1)
	376	*Tet(K)*:TPA	*S. warneri* strain 16A plasmid (CP031268.1)
C36	33	*BlaI*:*BlaR1*:*blaZ*:::transposase:tyrosine-type recombinase/integrase	*S. epidermidis* strain O47 chromosome (CP040883.1)
	59	*RadC*::*erm(A)*: *ANT(9)-Ia*:transposase: tyrosine-type recombinase/integrase:tyrosine-type recombinase/integrase:*RadC*::::recombinase	*S. aureus* strain BPH2056 genome assembly, chromosome (LR027874.1)
	81	IS431*mec* (IS257)::::*mecA*:*MecR1*	*S. aureus* strain Guangzhou-SAU071 chromosome (CP053183.1)
	91	relaxase:*MobC*:*cat(pC233)*	*S. aureus* plasmid pC223 (AY355285.1)
	93	relaxase:::*cat(pC221)*:::relaxase	*S. aureus* strain 08-028 plasmid (CP045437.1)
C38	42	recombinase:*CcrB*::::IS1182::*MecR1*:*mecA*::::IS6-like element(IS257)	*S. aureus* strain NZAK3, chromosome (LT009690.1)
	54	*erm(C)*:ErmCL	*S. aureus* strain 18082 chromosome
C40	31	*YycH*::::IS6-like element (IS257)::::*mecA*:*MecR1*	*S. epidermidis* strain HD66 chromosome (CP040868.1)
	61	*Erm(C)*:*ErmCL*:*RepL*	*S. aureus* strain 18082 chromosome (CP041633.1)
C81	30	*BlaI*:*BlaR1*:*blaZ*:::transposase:*XerC*:Tn554-related transposase A	*S. epidermidis* strain O47 chromosome (CP040883.1)
	144	Recombinase/integrase:Tn554:*ANT(9)-Ia*:*erm(A)*	*S. aureus* strain SR153 chromosome (CP048643.1)
	148	IS6-like element (IS257)::::*mecA*:*MecR1*	*S. aureus* strain Guangzhou-SAU071 chromosome (CP053183.1)
	160	*cat(pC221)*:::::*Erm(B)*	*S. pseudintermedius* strain AH18 chromosome (CP030374.1)
C119	12	recombinase:*BlaI*:*BlaR1*:*blaZ*:: type I toxin-antitoxin system	*S. epidermidis* strain SE95 plasmid (CP024439.1)
	204	*Erm(C):*ErmCL	*S. aureus* strain 18082 chromosome (CP041633.1)
C133	6	TPA:Tn554::IS1182:*blaZ*:*BlaR1*:*Bla*I	*S. epidermidis* strain NCCP 16828 chromosome (CP043847.1)
C135	70	resolvase:*CcrB*::::IS1182::*MecR1*:*mecA*	*S. aureus* strain ER03750.3 chromosome (CP030557.1)
	75	IS6:*blaZ*:*BlaR1*:*BlaI*:recombinase	*S. epidermidis* strain Z0118SE0260 chromosome (CP060794.1)

	175	*Fst*:::*msr(A)*:*Mph(C)*	*S. aureus* strain ER02243.3 plasmid (CP030478.1)
C137	31	IS6:*blaZ*:*BlaR1*:*BlaI*:recombinase	*S. epidermidis* strain SESURV_p1_1200 chromosome (CP043796.1)
C138	4	*BlaI*:*BlaR1*:blaZ::TN554:TPA	*S. epidermidis* strain NCCP 16828 chromosome (CP043847.1)
	37	*dfrG*:Insertion element (TPA)	*S. epidermidis* strain NCCP 16828 chromosome (CP043847.1)
C145	30	IS1182::*MecR1*:*mecA*	*S. aureus* strain NRS484 chromosome (CP026066.1)
	34	GNAT family N-acetyltransferase:APH(3')-IIIa:Peptide binding protein::*erm(B)*::antitoxin: peptide-binding protein: IS1216(IS6)::::recombinase	*E. faecium* strain VVEswe-R plasmid (CP041269.2)
	36	*Lnu(B)*::::*ant(6)-Ia*:sat1):ISEfm1	*E. faecium* strain Efm0123 plasmid (KR066794.1)
	54	GNAT family N-acetyltransferase:*APH(2'')-Ia*	*S. hominis* strain FDAARGOS_661 plasmid (CP054551.1)
C148	17	recombinase:*BlaI*:*BlaR1*:*blaZ*::type I toxin-antitoxin system	*S. cohnii* strain FDAARGOS_744 plasmid (CP054810.1)

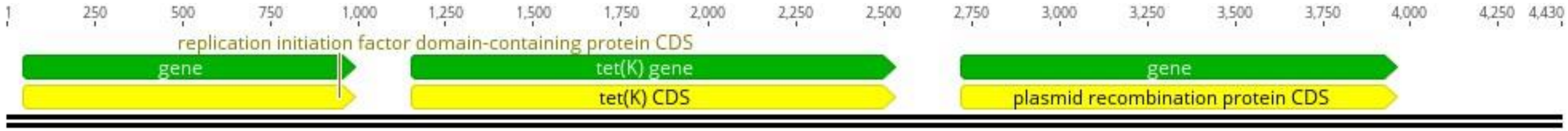
(a) C35

(I)



C35_contig 237

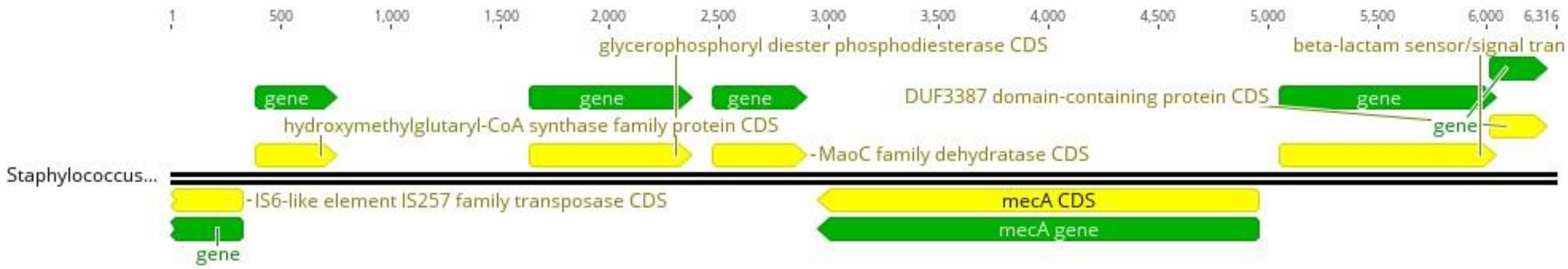
(II)



C35_contig 376

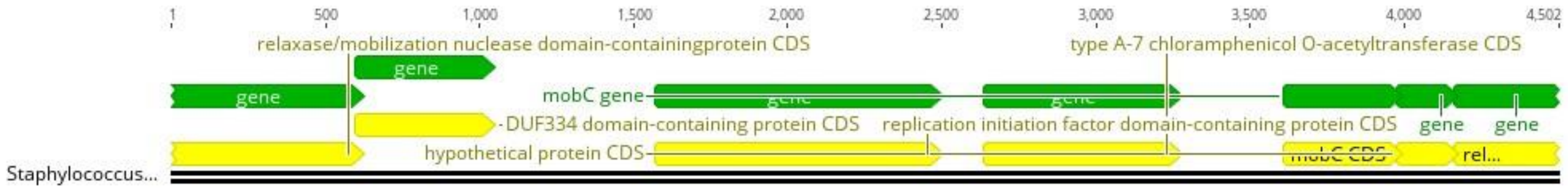
(b) C36

(I)



C36_contig 81

(II)

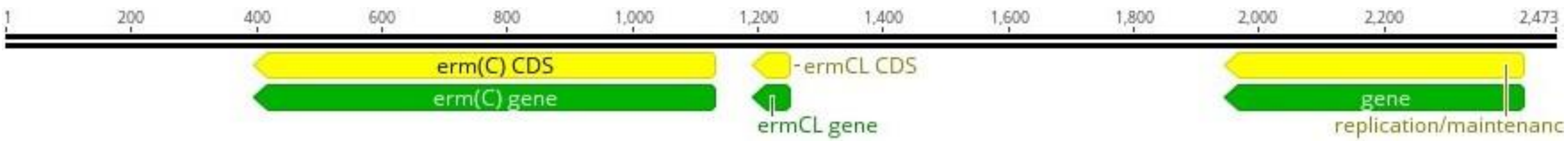


C36_contig 93

(c) C40

C40_contig 31

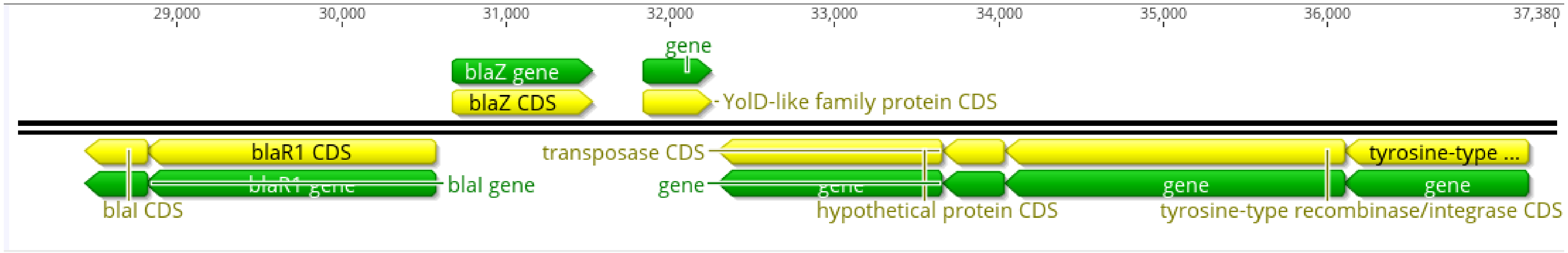
(I)



C40_contig 61

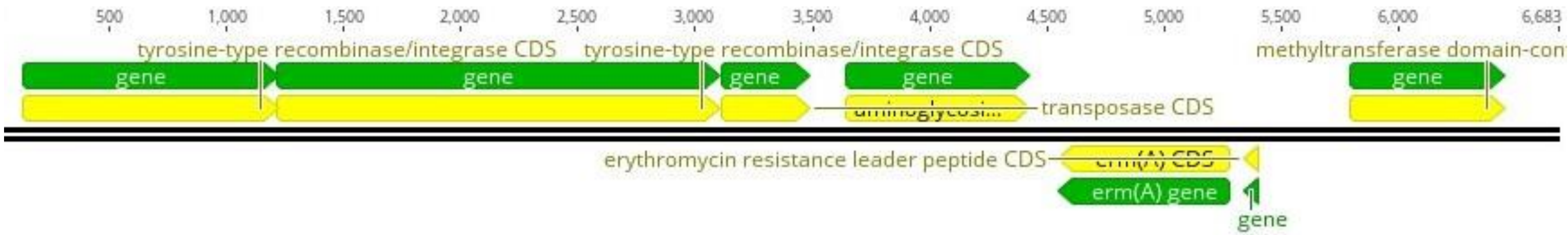
(d) C81

(I)



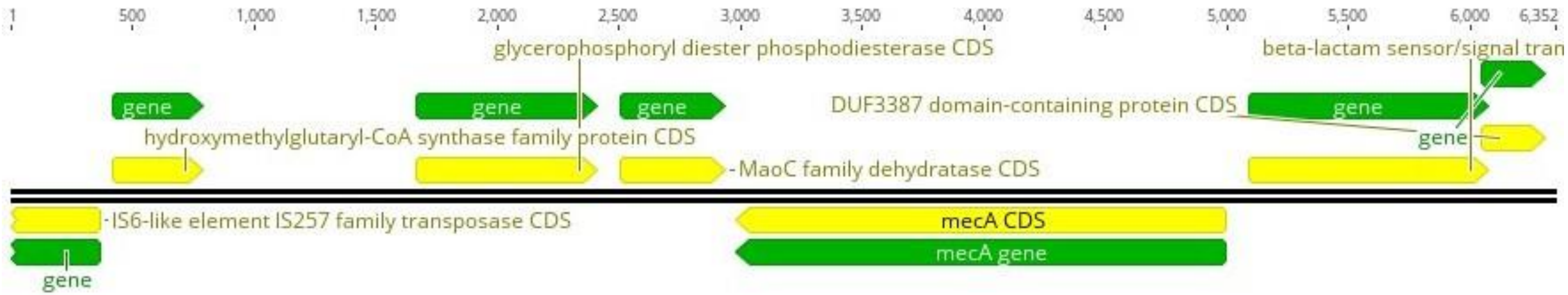
C81_contig 30

(II)



C81_contig 144

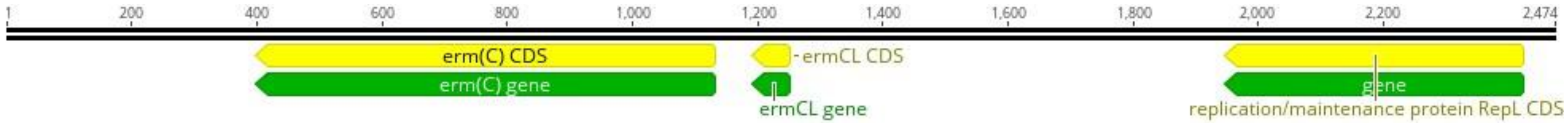
(III)



C81_contig 148

(e) C119

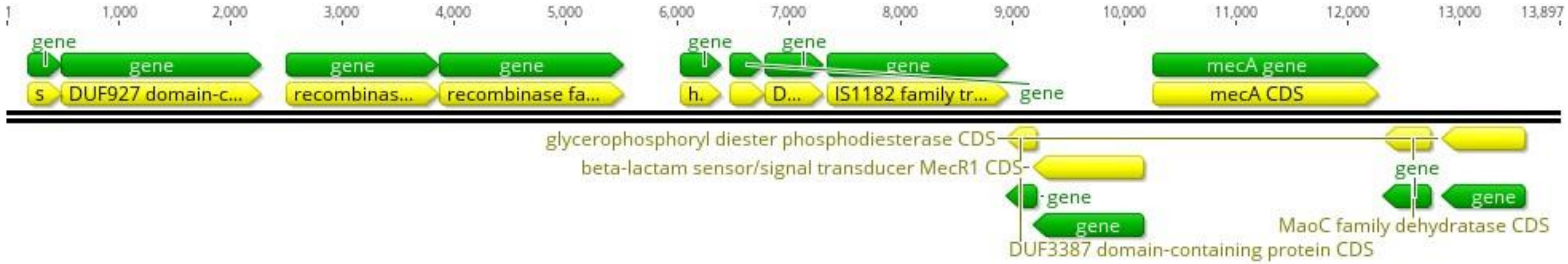
(I)



C119_contig 204

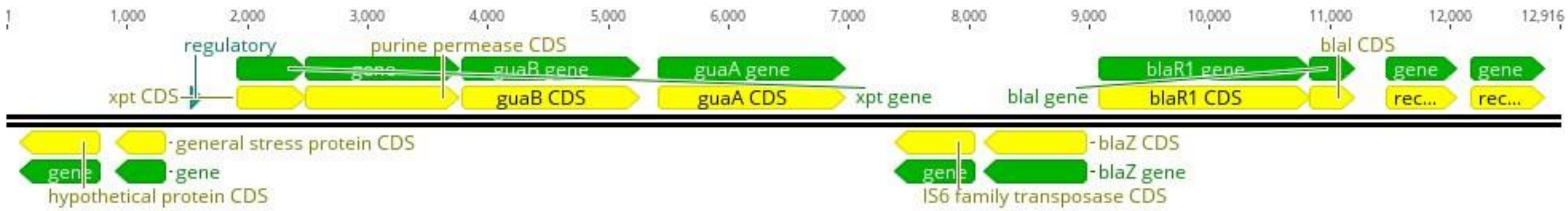
(f) C135

(I)



C135_contig 70

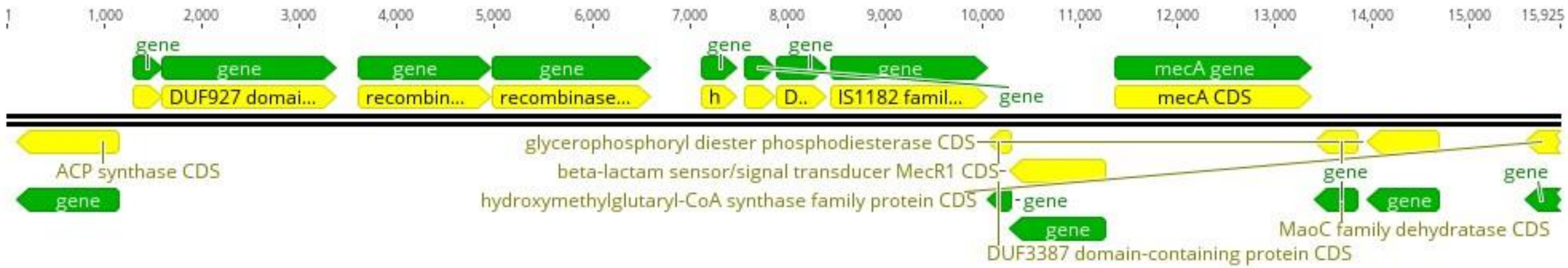
(II)



C135_contig 75

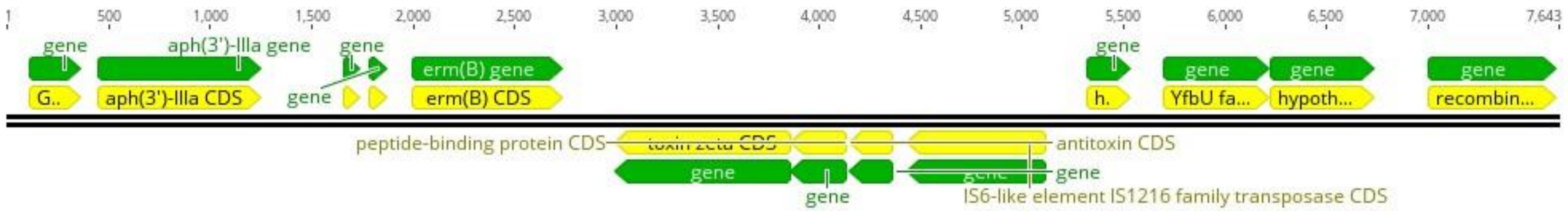
(g) C145

(I)



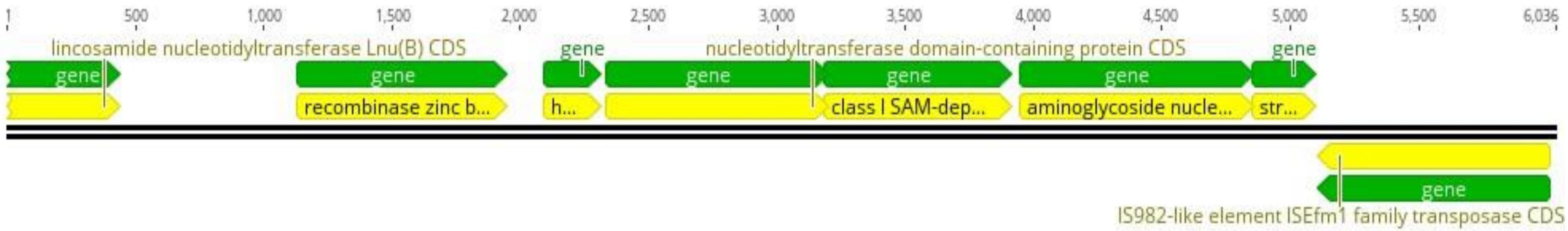
C145_contig 30

(II)



C145_contig 34

(III)



C145_contig 36

Figure 1 Genetic environment of resistance genes found in the MRSE strains. The genetic environments of genes were determined using the annotated GenBank files. Isolate numbers are represented by letters, while roman numerals indicate the various contigs of the individual genomes

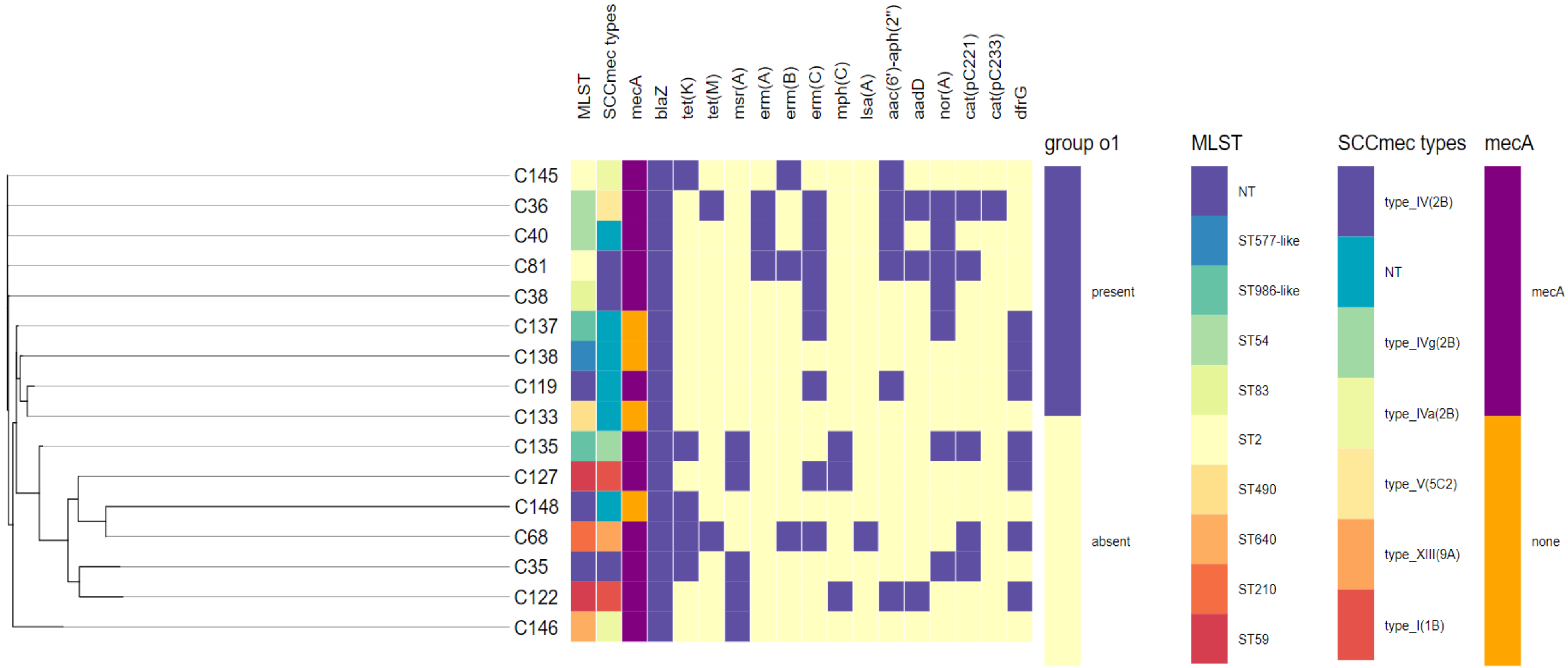


Figure 2a Whole-genome phylogenetic tree annotated with sequence type (ST) assignment, SCC*mec* types and antibiotic resistance carriage. Gene names above the annotation according to antibiotic class as follows (β-lactams: *mecA*, *blaZ*; tetracyclines: *tet(K)*, *tet(M)*; macrolide, lincosamide streptogramin B: *msr(A)*,*erm(A)*, *erm(B)*, *erm(C)*, *lsa(A)*, *mph(C)*; aminoglycosides: *aac(6')-aph(2'')*, *aadD*; efflux pump: *norA*; trimethoprim: *dfrG;* chloramphenicol: *cat(pC221)*, *cat(pC233).* The profile was generated using Phandango (https://jameshadfield.github.io/phandango/#/). Heat map at the middle indicates antibiotic resistance gene presence (purple) and absence (yellow). NT: Non-typeable

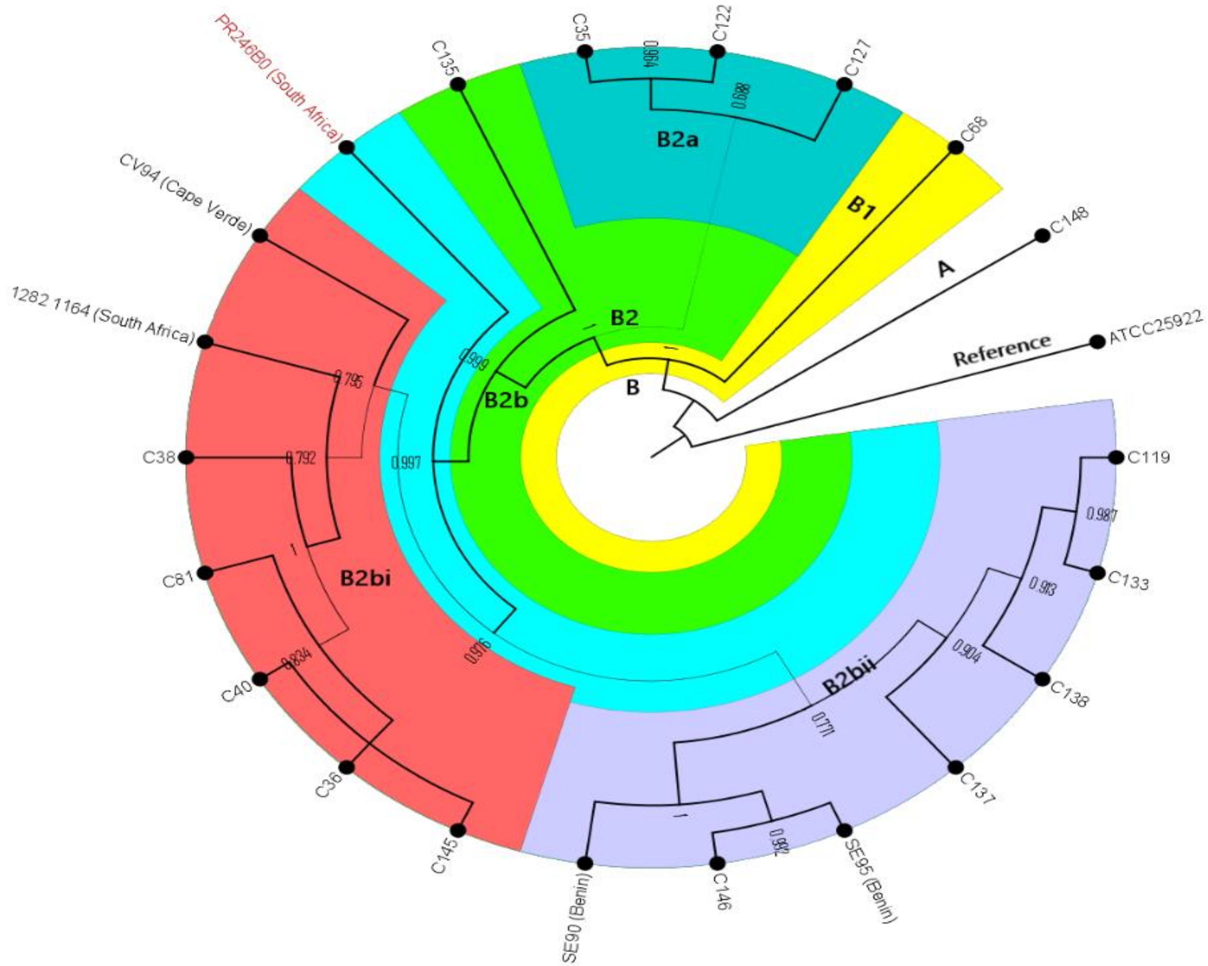


Figure 2b Phylogenetic relationship of *S. epidermidis* isolates in this study and African *S. epidermidis* (with their countries indicated) obtained from PATRIC. The tree consists of two main clades, A and B, defined by the branches. Clade B was further organised into sub-clades B1 and B2, which was further organised into B2a and B2b, and subsequently into B2bi and B2bii.

Table S1 Genome and assembly characteristics of sequenced MRSE isolates from clinical sources

Isolate ID	**Size (Mb)**	**GC%**	**contigs**	**No. of RNAs**	**No. of coding sequences**	***N_{50}***	***L_{50}***
C35	2.9	31.8	552	15	3185	6031	166
C36	2.8	32.0	177	75	53309	53309	17
C38	2.7	32.4	61	82	2657	91621	10
C40	2.5	31.8	53	63	2487	94191	9
C68	2.8	31.9	140	65	2834	82977	10
C81	2.8	31.9	268	68	2959	25474	37
C119	2.4	32.5	1331	34	3385	3259	146
C122	2.7	31.7	1267	45	3609	9443	65
C127	2.8	31.8	255	92	2922	31786	27
C133	2.4	32.0	22	62	2295	348106	3
C135	2.8	32.2	678	65	3171	9750	72
C137	1.9	32.1	141	61	1987	24369	23
C138	2.9	31.9	541	63	3156	20755	35
C145	2.6	32.2	103	90	2597	126613	7
C146	2.3	32.0	25	56	2188	171915	4
C148	2.5	32.5	117	68	2587	315043	3

Key: N50 = smallest contig of the size-sorted contigs that make up at least 50% of the respective assembly

L50 = number of contigs that make up at least 50% of the respective total assembly length

Table S2. A table showing the eBURST (Based Upon Related Sequence Types) analyses of the study sequence types with global curated STs in PubMLST database.

MLST (Isolate)	Type of clone	Closet global ancestry sequence type (ST)	Source
ST54	Similar [a]	ST54	Human, Animal
ST83	Similar [a]	ST83	Human
ST210	Similar [a]	ST210	Human
ST2	Similar [a]	ST2	Human, Environment
ST59	Similar [a]	ST59	Human, Animal
ST490	Similar [a]	ST490	Human
ST640	Similar [a]	ST640	Human
ST69 (SG6)	Similar [a]	ST69	Animal (Food), Human
ST155 (SLC2, TLC13, CM4)	Similar	ST155	Animal (Food), Human, Environment

ST297 (TLC1)	Similar	ST297	Human
ST1727 (NC3)	Similar	ST1727	Human
ST44 (AC1)	Single-Locus Variant (SLV) [b]	ST10, ST752	Animal (Food), Human
ST469 (CC6)	Single-Locus Variant (SLV)	ST162	Food
ST540 (AB1, TG1)	Single-Locus Variant (SLV)	ST4093	Human
ST1141 (NM11)	Single-Locus Variant (SLV)	ST10, ST744	Animal (Food), Human
ST7473 (NB12)	Single-Locus Variant (SLV)	ST10	Animal (Food), Human
ST6646 (CB1)	Satellite [c]	None	-
ST7483 (NB12)	Satellite	None	-

Table S3 Distribution of intact prophage regions among the MRSE strains

Sample code	Intact prophages	Region	Length (kb)	No. CDS	GC%	Phage	Resistance genes
C35	-	-	-	-	-	-	-
C36	2	1	34		28.44	PHAGE_Staphy_187	-
		2	30.8	30826	33.42	PHAGE_Staphy_StB20_like	-
C38	2	1	34		28.43	PHAGE_Staphy_187	-
		2	65		32.95	PHAGE_Staphy_StB20_like	-
C40	2	1	34	34053	28.44	PHAGE_Staphy_187	-
		2	41.6	41681	32.7	PHAGE_Staphy_StB20_like	-
C68	-						-
C81	3	3	30.4	30449	28.63	PHAGE_Staphy_187	-
		4	24.3	24394	34.24	PHAGE_Staphy_StB20	-
		5	21.6	21691	33.7	PHAGE_Staphy_StB20	-
C119	1	1	34.1	34102	36.47	PHAGE_Entero_vB_IME197	-
C122	1	1	42.7	42796	34.14	PHAGE_Staphy_StB12	-
C127	-	-	-	-	-	-	-
C133	1	1	50.5	50527	34.29	PHAGE_Staphy_StB12	-
C135	-	-	-	-	-	-	-
C137	3	1	44.8	44882	54.65	PHAGE_Salmon_SEN34	-
		3	17.5	17545	55.52	PHAGE_Klebsi_phiKO2	-
		5	40.9	40943	55.52	PHAGE_Staphy_StB20	-
C138	1	2	40.9	40943	33.98	PHAGE_Staphy_StB20	-
C145	3	1	52.2	52234	29.21	PHAGE_Staphy_187	-
		2	41	41071	32.54	PHAGE_Staphy_StB20_like	-
		4	35.6	35674	36.21	PHAGE_Entero_vB_IME197	-
C146	-	-	-	-	-	-	-
C148	-	-	-	-	-	-	-

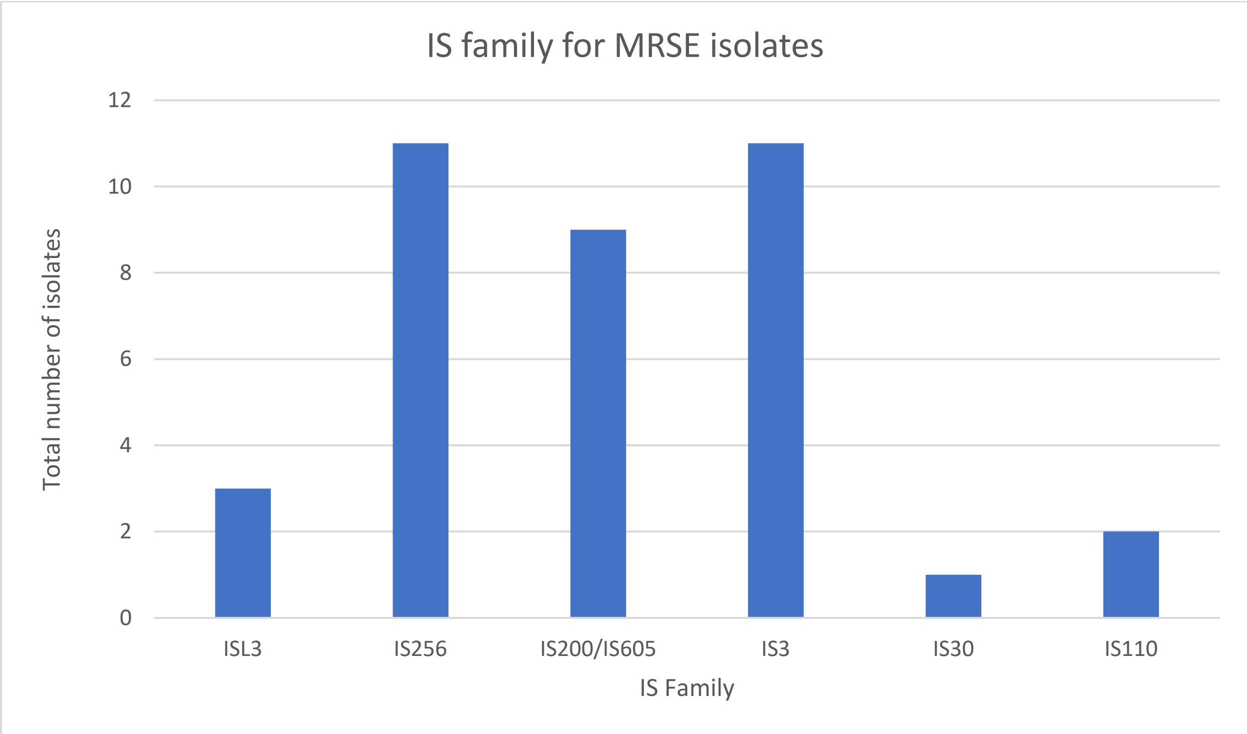


Figure S1 A bar chart depicting the total number of each predicted insertion sequence (IS) families in the MRSE isolates

CHAPTER 5 – CONCLUSION

The study investigates the molecular epidemiology and genomic profile of clinical coagulase-negative staphylococci from KwaZulu-Natal, South Africa.

5.1 Conclusions

The following were the main conclusions drawn from the study according to the study objectives

- To ascertain the incidence of CoNS from hospitals in the uMgungudlovu District of the KwaZulu-Natal Province from blood cultures routinely processed by the central microbiology laboratory using culture and biochemical techniques:
    - The overall incidence rate of CoNS was 68.5% (89/130), distributed across various hospital departments including ICU, NICU, paediatric ward, paediatric OPD, medical wards, emergency departments and surgical ward.
- To speciate the CoNS using the automated VITEK 2 system:
    - The distribution of CoNS species determined by VITEK 2 was *S, epidermidis* (19.1%), *S. haemolyticus* (16.9%), *S. hominis* ssp *hominis* (15.7%), *S. lentus* (14.6%), *S sciuri* (5.6%), *S. xylosus* (5.6%), *S. hominis* (4.5%), *S. succinus* (4.5%), *S. saprophyticus* (.4%), *S. gallinarum* (2.2%), *S. capitis* (2.2%), *S. lugdunensis* (2.2%), *S. auricularis* (1.1%), *S. arlettae* (.1%), *S. warneri* (1.1%).
- To determine the susceptibility profile of CoNS isolates against a CLSI-recommended antibiotic panel using the Kirby-Bauer disk-diffusion and MIC methods as appropriate:
    - The isolates displayed high resistance against penicillin (100%), cefoxitin (76.4%), azithromycin (74.2%), erythromycin (74.2%) and trimethoprim/sulphamethoxazole (68.5%).

- Isolates exhibited lower levels of resistance to gentamicin (2.2%), amikacin (4.5%), linezolid (4.5%), tigecycline (5.6%), nitrofurantoin (5.6%), teicoplanin (6.7%), and ceftaroline (10.1%).
- There was no resistance to vancomycin.
- Multidrug resistance was observed in 76.4% (68/89) of isolates with MAR indices ranging from 0.05 to 0.80. Fifty-one different resistance patterns were observed in MDR isolates depicting high resistance of the isolates to commonly used antibiotics.

* To identify and characterize antibiotic-resistance and virulence genes in CoNS, their associated MGEs and their genetic support/environment using whole genome sequencing and bioinformatics tools:
  - Sixty-three isolates were MRCoNS by PCR detection of the *mecA* gene. The *mecA* gene was borne on the SCC*mec* mobile genetic element (MGE).
  - Resistance genes conferring resistance to β-lactams (*mecA*, *blaZ*), tetracyclines [*tet(K)*,*tet(M)*], macrolide-lincosamide-streptogramin B antibiotic (MLS_B) [*erm(A)*, *erm(B)*, *erm(C)*, *msr(A)*, and *mph(C)*], trimethoprim-sulfamethoxazole (*dfrG*), aminoglycosides [*aac(6')-aph(2'')*, *aph(3')-III*, *aadD*], chloramphenicol [*cat(pC221)*, *cat(pC233)*], fosfomycin [*fosB*], were detected in isolates. There was generally agreement between phenotype and genotype.
  - Seven mutations were found in *gyrA*, 4 mutations in *parC*, but no mutations were detected in *gyrB*. We further found 2 mutations (S486Y and Y737S) in the *rpoB* gene.
  - Various virulence genes, including those involved in biofilm/adherence formation (e.g., the *ica* operon, *atl*,and *ebh*), enzymes (e.g. *geh*, *lip*, and *sspA*) and immune evasion (e.g., *adsA*,*capC* and *manA*) were present in isolates.

    - Other MGEs found included insertion sequences (including the virulence-associated IS256), plasmid replicons (mostly Rep10) and phages (mostly PHAGE_Staphy_StB20). Other MGEs such as transposons (Tn554) and IS257 were frequently found as part of the genetic support environment of resistance genes.
    - Mechanisms encoding defense against prophages (RMS) and the MGE ACME, were also identified.

- To determine the clonal relatedness and phylogeny of isolates using whole-genome sequencing and bioinformatics tools to compare and contrast study strains with others.
    - Phylogenetic analysis found two major clades, A and B, with clade B further divided into sub-clades, and that antibiotic resistance gene distribution did not coincide with clades. There were no associations of note between clades and hosptital/ward type.

5.2 Limitations

- The small numbers of CoNS isolates obtained from specific departments made it challenging to deduce statistically significant comparisons across the hospitals' departments.
- Considering the importance of identifying patients at risk of CoNS infection, the absence of detailed information such as the period of hospitalisation, previous antibiotic treatment, immune status of patients, clinical signs and symptoms and co-morbidities made it challenging to assess the true pathogenic potential of isolates recovered, even though WGS gave us important insights.

5.3 Recommendations

- Future studies should consider large numbers of isolates to explain trends observed with greater statistical significance.
- Future studies should be more patient-focused, with the provision of detailed patient-level data to assist in the assessment of other factors that contribute to pathogenicity/virulence and also to consider confounding factors.
- There is a need for surveillance of CoNS as emerging opportunistic pathogens.
- There should be increased education and awareness creation about antibiotic resistance and spread to decrease its occurrence, particularly in the clinical setting.

5.4 Significance of the research

Antibiotic resistance is an issue of global concern as the use of antibiotics continues to rise both in human and veterinary practice, leading to the development of multidrug-resistant infections, associated with increased morbidity and mortality. The high level of multidrug resistance observed and the rich repertoire of resistance genes, virulence genes and MGEs call for greater monitoring and evaluation in the South African health sector when dealing with CoNS.

Investigation into the resistance profile, virulence profile, pathogenicity, persistence, defense mechanisms, phylogenetic relationships and evolution provides vital insights into the genomic underpinnings of the behaviour of CoNS. The study points to the fact that there is a need for improved screening of both patients and healthcare workers, considering CoNS are abundant on the skin and mucous membranes. Thus, great caution should be exercised during invasive medical procedures as well as those that involve the insertion of medical devices.

There is scanty information concerning the role of CoNS in hospital-acquired infections, particularly relating to molecular epidemiology in Africa, with most studies reported from Europe and the USA. Thus, the study sought to describe the molecular and genomic epidemiology and mechanisms that make CoNS microorganisms of interest, using WGS. The use of WGS in this study provided critical information useful for the epidemiology of infection, diagnosis and typing, useful to arrive at important clinical and public health decisions.

APPENDICES:

APPENDIX I:

Ethical Approval

BIOMEDICAL RESEARCH ETHICS COMMITTEE (BREC) APPROVAL LETTER

UNIVERSITY OF KWAZULU-NATAL ™
INYUVESI YAKWAZULU-NATALI

01 September 2020

Mr Jonathan Asante (214584534)
School of Lab Med & Medical Sc
Medical School

Dear Mr Asante,

Protocol reference number: BREC/00001302/2020
Project title: Comparative Whole Genome Profiling of Pig and Clinical Coagulase-negative staphylococci Isolates: A One Health Comparative Study from Umgungundlovu District, South Africa.
Degree Purposes: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 01 September 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 26th August 2020, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_2_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 01 September 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 13 October 2020.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

APPENDIX II

Ethical approval-Amendment

BIOMEDICAL RESEARCH ETHICS COMMITTEE (BREC) AMENDMENT APPROVAL LETTER

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

07 January 2021

Mr Jonathan Asante (214584534)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mr Asante,

Protocol reference number: BREC/00001302/2020
Project title: Comparative Whole Genome Profiling of Pig and Clinical Coagulase-negative staphylococci Isolates: A One Health Comparative Study from Umgungundlovu District, South Africa.
Degree: PhD
<u>NEW TITLE OF STUDY:</u> 'Molecular and genomic profiling of clinical multidrug-resistant coagulase-negative staphylococci from the KwaZulu-Natal Province in South Africa.'

We wish to advise you that your application for Amendments (listed below) received on 17 December 2020 for the above study has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee.

The following Amendments on your application has been noted and approved:

- Noted that very few coagulase-negative staphylococci (CoNS) samples were recovered from the study on pigs, precluding comparison. The study thus took a purely clinical focus investigating the molecular epidemiology of CoNS from hospitals in the uMgungundlovu district routinely processed by the central microbiology laboratory.
- The title of the study has therefore changed to 'Molecular and genomic profiling of clinical multidrug-resistant coagulase-negative staphylococci from the KwaZulu-Natal Province in South Africa.'
- Noted that that the amendment is necessary as enough pig samples could not be obtained. The amendment will have no extra impact on patients.

This approval will be noted at the next BREC meeting to be held on 09 February 2021.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

APPENDIX III

CLASS ETHICAL APPROVAL

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

17 March 2017

Prof SY Essack
Department of Pharmaceutical Sciences
School of Health Sciences
essacks@ukzn.ac.za

Dear Prof Essack

Title: One Health approach to the containment of antibiotic resistance.
Degree: Non-degree
BREC Ref No: BCA444/16

CLASS APPROVAL

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application at a meeting held on 13 September 2016.

The study was provisionally approved by BREC pending appropriate responses to queries raised. Your responses dated 28 February 2017 to queries raised on 19 September 2016 have been noted and approved by the Biomedical Research Committee at a meeting held on 14 March 2017.

This approval is valid for one year from **17 March 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Pg. 2/...

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za

The following Committee members were present at the meeting that took place on 13 September 2016:

Prof V Rambiritch	Acting Chair - Pharmacology
Prof C Aldous	Genetics
Prof R Bhimma	Paediatrics & Child Health
Rev. S D Chili	External - Community member
Dr T Hardcastle	Surgery
Dr R Harrichandparsad	Neurosurgery
Mr H Humphries	Research Psychology and Public Health
Dr M Khan	External - Obstetrics and Gynaecology
Dr Z Khumalo	KZN Health (External) General Medicine
Prof TE Madiba	General Surgery
Ms T Makhanya	External - Community member
Dr S Paruk	Psychiatry
Dr D Singh	Critical Care
Prof C Rout	Anaesthetics
Dr T Sookan	Biokineticist
Prof D Wassenaar	Psychology (Acting Chair)

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

PROFESSOR JOYCE TSOKA-GWEGWENI
Chair: Biomedical Research Ethics Committee

APPENDIX IV

ETHICS CERTIFICATE

TRREE

Zertifikat

Certificado

Certificat

Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Clinical Trials Centre
The University of Hong Kong

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Jonathan Asante

a complété avec succès - has successfully completed

Introduction to Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

[redacted]

Release Date: 2020/02/07
CID : 5I1XVWdHGQ

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

FMH Continuing Education Program (5 Credits)
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European and Developing Countries Clinical Trials Partnership (EDCTP) (www.edctp.org) - Swiss National Science Foundation (www.snf.ch) - Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca/e/2891.html) - Swiss Academy of Medical Science (SAMS/ASSM/SAMW) (www.samw.ch) - Commission for Research Partnerships with Developing Countries (www.kfpe.ch)

[REV : 20170310]

APPENDIX V

Manuscripts submission confirmation

Submission confirmation from Antibiotics

[Antibiotics] Manuscript ID: antibiotics-1100241 - Submission Received

From: Editorial Office (antibiotics@mdpi.com)

To: lutherkinga@yahoo.fr

Cc: josante33@yahoo.com; hetszab@gmail.com; amoakodg@gmail.com; besterl@ukzn.ac.za; essacks@ukzn.ac.za

Date: Wednesday, January 20, 2021, 04:52 PM GMT+2

Dear Dr. Abia,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Antibiotics
Manuscript ID: antibiotics-1100241
Type of manuscript: Article
Title: Multidrug-Resistant Coagulase-Negative Staphylococci from the uMgungundlovu District of Kwazulu-Natal Province in South Africa: Emerging Pathogens
Authors: Jonathan Asante *, Bakoena A. Hetsa, Daniel G. Amoako, Akebe Luther King Abia, Linda A. Bester, Sabiha Y Essack
Received: 20 January 2021
E-mails: josante33@yahoo.com, hetszab@gmail.com, amoakodg@gmail.com, lutherkinga@yahoo.fr, besterl@ukzn.ac.za, essacks@ukzn.ac.za
Healthcare Infections, Healthcare Environment and Antimicrobial Resistance
https://www.mdpi.com/journal/antibiotics/special_issues/Infections_Antibiotics

You can follow progress of your manuscript at the following link (login required):
https://susy.mdpi.com/user/manuscripts/review_info/be2622081f7a1919adf278abdb7f539f

The following points were confirmed during submission:

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